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FACULTY OF GRADUATE AND
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Spatial Encoding of Artificial Flowers by Bumblebees (*Bombus impatiens)* :
The Contents of Memory

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Spatial Encoding of Artificial Flowers by Bumblebees (*Bombus impatiens*): The Contents of Memory

Dana L. Church

Thesis submitted to the
Faculty of Graduate and Postdoctoral Studies
In partial fulfillment of the requirements
For the PhD in Experimental Psychology

School of Psychology
Faculty of Social Sciences
University of Ottawa

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Direction du
Patrimoine de l'édition

395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file Votre référence
ISBN: 0-494-10959-9
Our file Notre référence
ISBN: 0-494-10959-9

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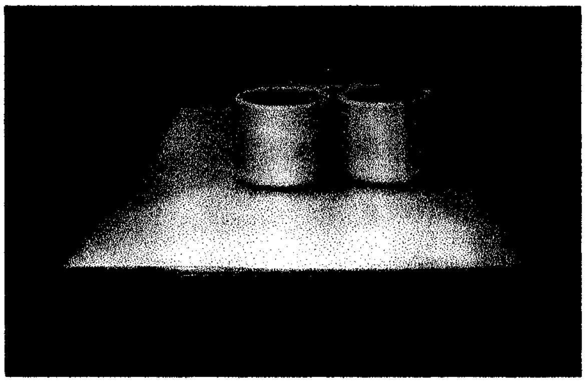


"But, for the point of wisdom, I would choose to know
the mind that stirs between the wings
of bees and building wasps."

- George Eliot, The Spanish Gypsy

To my parents, Andy and Theresa Church,

my partner, Mark Rathwell,

and in loving memory of Michelle Post—a friend and inspiration.

You are truly missed.

Table of Contents

Acknowledgements.......7
Abstract.......8

CHAPTER ONE

General Introduction.......9

CHAPTER TWO

Experiment 1

Method.......33
Results.......38
Discussion.......40

Experiment 2

Introduction.......42
Method.......44
Results.......45
Discussion.......47
Table 1.......52
Table 2.......53
Figure Captions.......54
Figure 1.......55
Figure 2.......56
Figure 3.......57
Figure 4.......58

CHAPTER THREE

Title Page....................59

Abstract....................60

Introduction....................61

Experiment 1

Introduction....................64

Method....................67

Results....................71

Discussion....................72

Experiment 2

Introduction....................73

Method....................74

Results....................76

Discussion....................78

General Discussion....................79

Acknowledgements....................82

References....................83

Table 1....................90

Table 2....................91

Figure Captions....................92

Figure 1....................93

Figure 2....................94

Figure 3....................95

CHAPTER FOUR

General Discussion........96

References........103

Appendix A........112

- Table 1........113
- Table 2........115
- Table 3........116
- Table 4........117
- Table 5........118
- Table 6........119
- Table 7........120
- Table 8........121
- Table 9........122

Appendix B........125

- Table 1........126

Appendix C........128

Acknowledgements

This thesis actually had its beginnings before I entered graduate school. Catherine Plowright was my supervisor during a summer undergraduate research position, during my fourth year honours thesis, and finally during my doctoral studies. Thank you, Catherine, for all of your guidance, support, patience, and encouragement over the years. You brought out the researcher in me that I didn't know was there!

Second, I would like to thank the members of my committee: Alain Desrochers, Sylvain Gagnon, and Pierre Mercier. Your feedback was always valuable and helpful. Special thanks also to the Natural Sciences and Engineering Research Council of Canada for funding my research throughout the course of my graduate studies.

Next, to my labmates, France, Ginny, Dalit, and Martine: thank you for being such constant sources of support, inspiration, stimulating conversation, and humour! I must also thank my family and friends, who were always there from the beginning—be it laughing at my bee stories, providing me with a wide spectrum of bee paraphernalia, but always cheering me on.

Mark, your patience, support, and confidence in me never waned. Thank you for withstanding one of the biggest tests of a relationship: graduate school!

And of course, I would finally like to thank the bees. For they, more than anyone, taught me the valuable lesson that in the quest to understand the other creatures with whom we share this Earth, great things come in small packages.

Abstract

A novel methodology allowed simultaneous investigation of three elements of bumblebee foraging behaviour: spatial encoding of flower position, landmark use, and scent marking. Bumblebees were presented with a row of artificial flowers in a flight cage, one flower offering reward (S+). Testing involved empty (i.e., unrewarding) flowers. In Experiment 1, flower covers presumed to be scent marked (old covers) were switched with one of the unmarked covers, or replaced with new, scent-mark-free covers. Results confirmed previous research: presence of old covers influenced response type rather than floral choice. Choice appeared to have been made using memory. In Experiment 2, the S- were moved during testing to change the relative position of the S+. New covers were used for half of the bees. The flower in the same absolute position (wrong relative position) as the S+ was consistently chosen, suggesting that the S- did not function as landmarks. Contrary to Experiment 1, old covers influenced flower choice. Experiment 3 replicated Experiments 1 and 2. Again, bees preferred absolute position, but results suggest relative position was encoded and influenced choice under certain circumstances. The effect of old vs. new covers continued to be inconsistent: choice means were higher with new covers, and probing often occurred on new covers. Finally, when flower array independent (FAI) information and memory for a flight vector were placed in conflict in Experiment 4, bees showed a bias for using FAI cues. Taken together, these experiments show that the definition of a landmark remains to be clarified, the role of scent marking remains elusive, and bumblebees showed a consistent bias for using FAI information to locate a goal. Contributions of this thesis are placed within the context of research with vertebrate species and natural bumblebee foraging behaviour.

CHAPTER ONE

General Introduction

Out of curiosity, researchers placed a ring of pinecones on the ground around the entrance to a wasp's nest. Soon a wasp left the nest, circled around the pinecone ring a few times, and then flew away. The researchers shifted the ring of pinecones so that it was beside the nest entrance but no longer surrounded it. The wasp returned and instead of flying into the nest entrance, she flew about the ring of pinecones. When the researchers moved the pinecones so that they again encircled the entrance hole, the wasp found the entrance hole and entered her nest. The wasp appeared to have become reliant upon the simple pinecone constellation for locating her home.

Tinbergen and Kruyt conducted this experiment in 1938 (Tinbergen, 1969). Besides providing some history about the study of invertebrate behaviour, it provides an example of how other animals, even as small as wasps, use features of the environment as guides during their day-to-day activities. Bumblebees, the focal species in this thesis, have good reason to remember and use features of the environment outside of their colony given the nature of their foraging behaviour. Two aspects of bumblebee foraging behaviour that are related to memory—scent marking and landmark use—will be the focus of this thesis. But first, below is a discussion of what is known so far about the steps bumblebees use in finding food, and how memory may be implicated in these steps. It is important to note that some of the research mentioned involves honeybees. Indeed, up to this point in time honeybees have generally been the species of focus in bee memory research while bumblebees have been relatively neglected. Although cross-species generalizations can be problematic, the findings from honeybees will be considered to at least put this thesis within a context. Important differences between honeybees and bumblebees will be noted where appropriate.

Various Expressions of Bee Memory During Foraging

Preamble: The life cycle of the colony in a nutshell. In temperate regions a bumblebee colony's lifespan stretches from spring to late summer. In spring queens emerge from hibernation and search for an abandoned vole, mouse, or chipmunk nest in or near the ground. Once such a home is found the queen collects nectar to feed herself, and pollen that she will use to place a wax covering over her eggs. She then begins laying eggs and spends the next few weeks incubating them, occasionally leaving the nest to collect more nectar and pollen. The eggs develop into larvae, pupate, and hatch as worker bees. Once worker bees have emerged the queen remains within the nest and continues laying eggs and incubating her brood. Workers now take over the job of collecting pollen and nectar. Pollen is used mostly to cover and feed the eggs and larvae, whereas the queen and workers consume the nectar. Workers also aid the queen in feeding and incubating the larvae. Worker bees generally live for only one to two weeks, but the queen's continuous production of eggs ensures new workers emerge to replace those who have expired (Goulson, 2003; Heinrich, 1979a).

Near the end of the summer and the colony's cycle, the queen begins to lay eggs that will hatch into new queens and others that will hatch into males (all worker bees are females). Mature queens and males leave the colony so that males can find a mate and the queens can be fertilized. In the meantime the old queen and remaining workers die off. Once a new queen is fertilized she burrows into the ground to hibernate for the winter. In the spring she emerges, ready to start the cycle anew (Goulson, 2003; Heinrich, 1979a).

To a flower patch and back again. Worker bees therefore bear the responsibility of continuously supplying nectar for the queen and other workers so that the colony can survive long enough to produce new queens and males for gene dispersal (Goulson, 2003; Heinrich, 1979a). When a novice worker bee embarks on her initial foraging trip, after leaving the colony her flight path may veer in various directions but she continuously calculates her distance traveled from the colony using the amount of image motion over her retina (also referred to as optical flow; Collett & Collett, 2000; Esch & Burns, 1995; Srinivasan, Zhang, Lehrer, & Collett, 1996). Using the sun and the polarization pattern of the sky, she also keeps a running tally of her compass direction from the colony (Chittka, Williams, Rasmussen, & Thomson, 1999). After finding and collecting food the bee adds up the individual flight vectors she followed to get to where she is, resulting in a trajectory that can lead her straight back to the colony. This process is referred to as path integration or dead reckoning (Collett, 1998; Collett & Collet, 2000; Etienne, Berlie, Georgakopoulos, & Mauer, 1998; Fry & Wehner, 2002). Such "goal vectors" are computed both for the food source and for the colony, and can be recalled and used on subsequent foraging trips (Collett, 1996).

Although dead reckoning is usually a successful means of navigation, it carries two major problems. First, error can accumulate in the calculation of the final vector, as happens if the animal turns around while collecting food (Collett, 1996; Shettleworth, 1998). Second, if the bee is suddenly displaced by side winds or other means, following the vector will now lead to the wrong location. To compensate for these limitations, bees select objects in the environment that will act as landmarks (Cheng, 2000; Collett, 1998; Menzel, Gumbert, Kunze, Shmida, & Vorobyev, 1997). Landmark learning has been found to take place both as a bee arrives to the goal and as she departs, although learning during departure has lead to stronger performance (Couvillon & Bitterman, 1992).

Collett and Collett (2000) and Fry and Wehner (2002) suggest that the more times a bee takes a particular route it is more likely she will rely on landmarks rather than dead reckoning. Dead reckoning becomes a "back-up" system utilized if physical information cannot be used or becomes unreliable (Collett & Collett, 2000). However, Cheng (2000) presents a model of honeybee navigation that involves both memory for landmarks and memory for goal vectors. He argues that bees follow a sequence of steps when finding a goal. First, bees recall and travel along a goal vector that will lead them to the vicinity of the target. Next, they locate a familiar landmark and fly towards it. The bee then needs to leave the landmark and head towards the goal; thus, the third step is striking a vector from the landmark to the goal. Finally, once near the target, the bee engages in "image matching": positioning herself such that surrounding landmarks are placed at the correct positions on her retinas, indicating the direction and distance she must fly to finally reach the goal.

Although testing of Cheng's (2000) model is pending, noteworthy is that this and other research to date suggest that experienced foragers possess a "toolkit" of mechanisms upon which they rely when returning to a place. As outlined above, this includes memories for vectors, landmarks, and retinal images (e.g., Collett, 1998, Collett & Collett, 2000; Fry & Wehner, 2002; Menzel et al., 1997). The nature of these memories and how they become established in the bee's brain remain to be explained.

Species differences after homecoming? The events that occur after a worker bee returns to the colony with a load of food has been argued to differ between honeybees and bumblebees. Specifically, honeybees have been observed to perform a “waggle dance”: once back in the colony the successful forager waggles her abdomen back and forth, vibrates her wings, and moves in a figure-eight pattern. Meanwhile, other bees in the colony surround the dancer and touch her with their antennae. It has been hypothesized that specific features of the dance (e.g., the angle of the dancer’s body from the vertical, the frequency of vibrations of the dancer’s abdomen) tell the surrounding bees the direction and distance from the colony to the source of food. Upon leaving the colony these recruits can then use this communicated information to find the food themselves. This process has been coined the “dance language” hypothesis (von Frisch, 1967).

Conversely, with bumblebees this dancing behaviour is absent. It appears that returning bumblebee foragers simply deposit the collected food into storage cells in the colony, leaving other workers to find food locations on their own (Heinrich, 1979a). However, Dornhaus and Chittka (1999) speculate that when a worker bumblebee returns to the colony with a nectar load, the odour from this bee may stimulate experienced foragers to embark on a foraging trip. It may also communicate to nest mates inexperienced with foraging the flower species from which they may currently obtain food. Some researchers vehemently argue that this odour hypothesis has not been properly tested with honeybees, and the popular honeybee dance language hypothesis only weakly accounts for the data (Gould, 1976; Wenner, 2002). Additionally, it is possible that foraging honeybees use both detection of odour and communicated information from returning foragers (Shettleworth, 1998).

Regardless, the role of memory in honeybee dance behaviour remains to be explored. In addition, if the dance language hypothesis is refuted this would suggest that honeybees and bumblebees may be more similar in terms of post-foraging processes than was previously thought.

What constitutes a landmark for a bee? As outlined above, landmarks play an important role in spatial memory when bees return to a place. Curiously, there are no conclusions as to what exactly constitutes a landmark for a bee. Researchers have been quite creative in selecting objects that bees can use as landmarks. Examples include wooden blocks (Brown, Moore, Brown, & Langheld, 1997; Huber, Couvillon, & Bitterman, 1994), curved shields (Huber et al., 1994), large coloured tents (Chittka & Geiger, 1995a; Chittka & Geiger, 1995b; Chittka, Geiger, & Kunze, 1995), coloured cylinders (Cheng, Collett, & Wehner, 1986; Cheng, Collett, Pickhard, & Wehner, 1987), a mountain (Menzel, Geiger, Joerges, Müller, & Chittka, 1998), and a line of trees (Dyer & Gould, 1983). But what is it about all of these objects that allows them to be classified as landmarks?

First, colour appears to be important: honeybees have been found to discriminate between two different patterns of colours formed by two groups of landmarks, and bees tend to prefer using landmarks whose colour(s) contrast with the colours of the surroundings. Colour may be an important characteristic because, at least to the human eye, unlike size or shape the colour of the landmark does not change as the bee flies closer or farther away (Cheng et al., 1986).

Second, landmarks that are found close to the goal seem to be weighed more heavily by a foraging bee than landmarks that are further away from the goal (Cheng et al., 1987). Cheng and his colleagues exposed bees to four landmarks in a square arrangement surrounding a nectar source, and two landmarks immediately adjacent to the source. When the nectar was removed and the immediately adjacent landmarks were displaced at a different location, bees spent more time flying near these landmarks than the landmarks in the square arrangement. These authors concluded proximity to the goal seems to be important in a bee's choice of landmark.

In sum, the current conclusion is that prominent objects close to the goal will be more effective as landmarks (Cheng, 2000; Chittka et al., 1995). However, what constitutes "prominent" and "close" for a bee has still not been clearly defined. In this thesis the unrewarding flowers are the closest and most prominent objects near the goal. Specific manipulations will determine whether bumblebees encode the position of the rewarding flower relative to the positions of the unrewarding flowers. If so, the unrewarding flowers function as landmarks. Ergo, other flowers can be used as landmarks, and their appearance does not have to be different from that of the chosen flower.

Flower memories. On her first foraging trips, after a period of sampling patches of different flower species, the bumblebee will generally select two species with which she will restrict her future foraging. On each trip she will devote most of her food collection to one patch (the "major" patch), while visiting a smaller number of flowers from a second patch (the "minor" patch). This behaviour has been aptly termed "majoring" and "minoring." The minor patch may be thought of as a back-up, since depletion of the major's nectar due to other foraging individuals, or decreased floral density of the major's patch, will result in the bee devoting more time at the minor patch and less time at the major patch (Heinrich, 1976; Feinsinger, 1983). Restricting herself to these major and minor patches reduces search time and guarantees successful nectar and pollen loads on each trip. This is especially important because worker bees must collect food not only for themselves, but for the queen, other worker bees, and larvae in their colony (Heinrich, 1979a). Another advantage to this strategy is that individual worker bees within any one colony will major and minor in different plant species resulting in a broad diet for the queen and her brood (Feinsinger, 1983).

During the selection of major and minor patches, bees show preferences for flowers of certain colours (Giurfa, Núñez, Chittka, & Menzel, 1995; Simonds & Plowright, 2004), odours (Abramson, Aquino, Azeredo, Filho, & Price, 1997; Pernal & Currie, 2002), patterns (Lehrer, Horridge, Zhang, & Gadagkar, 1995), and nectar yields (Fülöp & Menzel, 2000; Waddington, Allen, & Heinrich, 1981). Although these preferences for certain colours and patterns appear to be unlearned in bumblebees (Simonds & Plowright, 2004), given an artificial situation they are capable of associating reward (nectar) with sources that may vary along any one or more of these dimensions. For instance, they can associate reward with different colours (Brown, McKeon, Curley, Weston, Lambert, & Lebowitz, 1998), odours, and colour-odour compounds (Bitterman, 1996). As to *how* the appearance of rewarding flowers are remembered, there is debate over whether bees store a photograph or template of the rewarding pattern in memory (Gould, 1985), or remember certain features of the pattern (Efler & Ronacher, 2000). In addition, after learning to associate various flower parameters with reward, bees may weigh these parameters differentially: Ney-Nifle, Keasar, and Shmida (2001) found that when both colour and location functioned to differentiate flower patches, bees tended to use the location cue rather than the colour cue.

Bees' memory for flowers has been tested using a paradigm similar to the radial arm maze used with rats (Olton & Samuelson, 1976). Specifically, these studies sought to determine whether or not bees would avoid revisiting food sites they had recently depleted. For instance, Brown and Demas (1994) used a piece of plywood containing six holes ("cells"), each filled with sucrose solution. To differentiate the cells a distinctly coloured wooden block was placed beside each one. Individual honeybees were allowed to visit three different cells in the matrix, a "visit" being defined as "a bee's entering a cell through the corresponding hole, so that at least its head and thorax were inside the hole" (Brown & Demas, 1994, p. 346). After the bee made three visits she was captured in a small plastic vial, the first apparatus was removed, and it was replaced with a second, identical apparatus. Introducing this second apparatus was meant to eliminate any scent cues the bees left behind. The three cells that were not previously visited by the bee contained a drop of sucrose, whereas the cells that were previously visited contained water (which is unrewarding to bees). The bee was released and her sequence of cell choices on this new apparatus was observed. Correct visits were to cells on the second apparatus that were not visited on the first apparatus (Brown & Demas, 1994).

As expected, bees showed a tendency to avoid revisiting locations previously depleted of nectar (i.e., they used a win-shift strategy), and Brown and Demas (1994) concluded that the honeybees used spatial memory to do so. However, two methodological problems must be noted. First, results showed that bees often ceased foraging before visiting all remaining cells: "bees failed to make a second choice on the second apparatus during 19% of the trials, and failed to make a third choice during 40% of trials" (Brown and Demas, 1994, p. 348). The poor rate of response may have been caused by the disruption from capturing and releasing the bees into the second apparatus (Brown & Demas, 1994; Burmeister, Couvillon, & Bitterman, 1995). Second, it was still possible for bees to leave behind a scent cue on visited cells of the first apparatus since bees were allowed to visit more than one cell (Burmeister et al., 1995). Detection of the scent cue could account for the tendency to avoid recently visited cells.

To improve upon Brown and Demas's (1994) study, Burmeister et al. (1995) devised a way to test memory in honeybees using three artificial flowers (conical dishes) that were placed inside an enclosure. A drop of sugar solution was placed in the center of each flower. While the bee was drinking from the second flower it selected, the experimenter replaced the first flower it chose with a fresh flower containing a drop of water. The bee now had the choice of returning to the first flower it chose, or visiting the third, yet unvisited flower. Three arrangements of the flowers were used: a row, a triangle, and then a fourth flower was added to make a rectangle.

Contrary to the results of Brown and Demas (1994), bees tended to return to flowers from which they had just fed (i.e., they used a win-stay strategy). Burmeister et al. (1995) concluded that their results show no evidence for spatial memory in bees. However, after probing deeper into the bees' win-stay strategy, Isnec, Couvillon, and Bitterman (1997) concluded that memory is indeed used: "Honeybees, like rats, seem to remember a rewarded location recently visited, but tend to return to it rather than, like rats, avoid it" (p.165). In a similar vein, Demas and Brown (1995) found that although their bees had a predisposition for a win-shift strategy they could be trained to use a win-stay strategy, suggesting "honey bees can flexibly adapt to the contingencies controlling the outcome of revisits to locations recently depleted of food" (p. 1045).

In sum, the bees in all of these experiments differentiated between visited and unvisited flowers using some kind of information. How this information was used resulted in different behaviour between studies: Brown and Demas (1994), and more recently Brown and colleagues (1997), found that bees *avoided* revisiting flowers (win-shift), whereas Isnec et al. (1997) found that bees is *returned* to them (win-stay).

Interestingly, one reason why these research groups obtained conflicting results is because different amounts of nectar were given to the bees. For a honeybee, feeding to repletion requires approximately 50 μl of nectar (Burmeister et al., 1995). Across experiments, Brown and Demas (1994), Demas and Brown (1995), and Brown et al. (1997) filled each rewarding cell of their apparatus with 2-5 μl of sugar solution. On the other hand, Burmeister et al. (1995) and Isnec et al. (1997) placed 10 μl of sugar in the rewarding dishes, which is double, or more than double, the amount of reward used by Brown and his colleagues. With this in mind, consider a study by Greggers and Menzel (1993) on honeybees' memory for nectar flow rates of flowers. They presented honeybees with four artificial flowers, with flow rates differing between flowers and varying from 0.0625 to 0.5 μl/min. Bees adjusted their foraging speed such that they foraged faster on flowers with a higher flow rate, and were more likely to show win-stay behaviour on high-flow-rate flowers and win-shift behaviour on low-flow-rate flowers. Indeed, "the rule, 'return to the food source after a feast' is a strong rule, not depressed by negative experience even after a large number of learning trials" (Greggers & Menzel, 1993, p. 28). Thus, if a honeybee considers 10 μl to be a feast and 2-5 μl to be a fast, this would explain why win-stay behaviour was observed by Burmeister et al. and Isnec et al. and win-shift behaviour was found by Brown and his colleagues. If this is true, replication with the reward volume used by the other group should reveal opposite behaviour to what was originally obtained.

The appearance of both win-shift and win-stay behaviour in their experiments lead Greggers and Menzel (1993) to conclude that bees used two types of memory when remembering flowers: (1) short-term memory for the reward just experienced at a flower; and (2) long-term memory for the individual flowers within a patch. How these memories interact remains to be understood. Since odour and colour of flowers were controlled for in this study, individual flowers were remembered by their location. Thus, spatial memory appears to play an important role in the foraging behaviour of bees--at least in laboratory environments. The evidence above from the work with radial arm maze analogues also supports this, and the preference Ney-Nifle and colleagues (2001) found for bees to use location over colour cues. The aim of this thesis is to go further by investigating *how* an array of artificial flowers is encoded in memory.

The Separate Story of Scent Marks

All of the experiments on bee spatial memory described above attempted to control for scent cues that bees may have left behind on visited flowers. It is implied that these scent cues are a separate, "non-memory" way for bees to select flowers. Below is a brief overview of the research on scent marking, followed by suggestions that scent marking may in fact be a form of memory.

When foraging on a patch of flowers, bumblebees and honeybees have often been seen to "hover in front of a flower, sometimes briefly touching the corolla with their antennae or legs, and then depart without probing the flower structure" (Goulson, Chapman, & Hughes, 2001, p. 670). Upon inspection these rejected flowers contain on average less nectar than the flowers that the bees have probed (Corbet, Kerslake, Brown, & Morland, 1984; Heinrich, 1979b; Kato, 1988; Wetherwax, 1986). How do bees assess nectar volume from afar? Depending on the structure of the flower species, nectar content may be directly visible to the bee (Kevan, 1976; Thorp, Briggs, Estes, & Erikson, 1975; Thorp, Briggs, Estes, & Erikson, 1976). Nectar volume may also be assessed by the scent of the nectar itself, the scent of fermentation products from yeasts in the nectar, and/or humidity gradients surrounding the flower (Crane, Williams, Hollands, & Tucknott, as cited in Goulson et al., 2001; Heinrich, 1979b). Evidence for these hypotheses, however, is lacking.

One proposed nectar-detection mechanism that has attracted research attention is scent marking: after visiting a flower the bee leaves behind a chemical, thought to originate from tarsal glands, which flags that flower's reward value. Evidence of scent marking has been found with both honeybees and bumblebees, in both laboratory and field settings. In some instances scent marks attracted bees to flowers, whereas in others they acted as repellants (Cameron, 1978, 1981; Giurfa & Núñez, 1992; Giurfa, Núñez, & Backhaus, 1994; Goulson et al., 2001; Goulson, Hawson, & Stout, 1998; Kato, 1988; Schmitt & Bertsch, 1990; Schmitt, Lübke, & Franke, 1991; Stout, Goulson, & Allen, 1998; Wetherwax, 1986; Williams, 1998). Reasons for these inconsistent results are unknown. It is also unclear whether bees leave scent marks at every flower visit, and whether they can perceive a visual and/or tactile dimension of these marks. The only consistent finding is that chemically analyzed flower surfaces have shown traces of chemicals after a bee visit, and these chemicals have subsequently influenced bee behaviour (Schmitt et al., 1991).

The function of scent marking is controversial. Some researchers claim scent marks play an attractant role to indicate to the scent-mark-depositing bee, and perhaps other bees, the rewarding value of a flower (Cameron, 1978, 1981; Schmitt & Bertsch, 1990). Cameron (1978, 1981) found that for bumblebees, scent marks functioned as a "to-probe-or-not-to-probe" cue: once at a flower, the bee will probe the flower if it had been previously probed and presumably scent marked. If the flower had not been previously probed she would not probe it herself. However, Thomson and Chittka (2001) argue that leaving an attractant on a flower would not be advantageous if it allowed competitors to detect and exploit the resource. It would make sense, though, if the scent mark attracted worker bees from the same colony. Nonetheless, encounters with nest mates outside of the colony are quite infrequent because: (1) bumblebees from any one colony return to different flower patches; (2) these flower patches are often widely spaced; and (3) the ratio of active foragers to the total number of workers in a colony is low. Attractants would only be beneficial if the regular visitors to the plant gained more rewards than casual visitors who visit less often. In sum, if bees indeed leave attractive scent marks on flowers, their exact function remains to be explained.

Alternatively, researchers who have found a repellent effect of scent marks argue that they function to identify recently drained flowers so bees can avoid them (Giurfa & Núñez, 1992; Giurfa et al., 1994; Goulson et al., 2001; Goulson et al., 1998; Kato, 1988; Stout et al., 1998; Wetherwax, 1986; Williams, 1998). Presently the scent marking research lacks chemical analyses to determine whether scent marks can vary in chemical composition--and as a result be interpreted as an attractant or a repellant--or whether the same scent can be interpreted differently (Chittka et al., 1999). Thomson and Chittka (2001) suggest these possibilities:

> It is equally possible that tarsal secretions are used as an adherence of bee feet to flowers, and are used as scent marks only as an epiphenomenon: bees might use the scent marks as repellents if the flowers are known to refill slowly, and as attractant if they remember the flowers as having high refill rates. (p. 207)

Using scent marks as a discriminative cue would require some form of memory, which is an exciting possibility considering that up to this point in time scent marking and memory have been studied separately. Scent marking has thus far been treated as a physical phenomenon not part of the bee memory system: a variable to be controlled for in memory studies. This thesis addresses this issue and presents a novel methodology that allows bee spatial memory and scent marking to be studied simultaneously.

Because the present study focuses on the performance of bumblebees in a laboratory setting, below is a more detailed account of the scent marking research that has been conducted with bumblebees in the lab.

Laboratory research with bumblebees. Cameron (1978, 1981) was the first to conduct research on scent marking by bumblebees in the laboratory and to claim that they leave behind a chemical on flowers. She presented bumblebees with four artificial flowers on the bottom of a small flight cage, each flower occupying a corner of a square arrangement. Each flower was made of a square piece of Plexiglas with a hole in the center that led to a refillable nectar well. Nectar wells were drilled in different spots of the floor of the cage so that the positions of the flowers could be varied. First, only one flower was made to offer a 1 μl reward of 50% sugar solution at each visit while the other three were unrewarding. Although the bees visited both types of flowers they only probed the rewarding flower. Second, after a bee visited the rewarding flower its position was switched with one of the unrewarding flowers. Bees probed both of these flowers above chance levels but not the two never-rewarding flowers. Finally, when the rewarding flower was washed with pentane, it no longer received preferential probing. Based on these results Cameron concluded that the bumblebees left behind a scent mark on the rewarding flower, and this scent mark functioned as a "probe-or-not-to-probe" cue.

Extending this research, Schmitt and Bertsch (1990) presented bumblebees with an array of 16 artificial flowers on a square platform. Each flower consisted of a plastic disc with a capillary tube underneath. The level of nectar in the capillary tube could be regulated such that for rewarding flowers, the nectar was at a level reachable by the bees, and delivered about 1 μl of sugar solution. For unrewarding flowers the nectar was unreachable. They observed the bees' behaviour when the plastic discs of rewarding and unrewarding flowers were exchanged and also after a 20h delay. Responses were classified as approach, land, or probe. Bees were found to probe flowers only if the flower had a plastic disc from a previously visited rewarding flower. This effect persisted even after the 20h delay. For flowers that did not have a plastic disc from a previously visited rewarding flower, bees simply approached the flower or landed on it without probing. A drop in foraging efficiency was also found when scent marks presumed to be on the flowers were removed. Thus, similar to Cameron's (1978, 1981) findings scent marks functioned as a "probe-or-not-to-probe" cue, but in addition they were found to have low volatility and their presence functioned to increased bumblebees' foraging efficiency.

Akin to bee spatial memory studies that included a control for scent marking, the scent marking studies described above included a control for spatial memory. It was not considered that some form of interaction might exist between the "probe-or-not-to-probe" effect of scent marks and memory for rewarding flower position. This thesis will include several methodological adjustments that will allow both phenomena to be studied at once. First, the order in which flowers are selected during testing will be considered. Each bumblebee's first test choice will be observed since this may be most indicative of memory for position of reward. Cameron (1978, 1981) and Schmitt and Bertsch (1990) only reported choice frequencies and not the order in which flowers were selected. Second, bees will be presented a test situation in which all scent marks are completely eliminated. This will be achieved by using completely new, unused flower covers. Complete elimination of scent marks is questionable in previous research since procedures often state the flowers were washed, leaving open the possibility of traces of scent marks remaining on the flowers. In this thesis, with absolutely no traces of scent marks available, bees would have to rely on memory for position of reward if they are to choose the flower that is in the same position as the rewarding flower in training. Finally, unlike procedures used in the bee memory studies discussed earlier, probing behaviour will be observed. Brown and Demas (1994), Burmeister et al. (1995), Isnec et al. (1997), and Brown et al. (1997) used apparatuses such that to obtain nectar, bees had to enter a hole or conical dish such that their head, at the very least, was occluded. This thesis will use an apparatus that allows an observer to see both flower choice and behaviour once on the flower.

Scope and Synopsis of This Thesis

In sum, three questions envelope the present study: (1) What is the definition of a landmark from a bee's perspective? (2) How does a bumblebee spatially encode a goal? (3) Is there an interaction at play between bumblebee spatial memory and use of scent marks? Unfortunately it is unrealistic to obtain complete answers to all of these questions through this thesis. Nevertheless, some partial answers have been gleaned by narrowing the scope of the questions and the problem space. Specifically, the bees' foraging environment was restricted to an array of two to three artificial flowers in a flight cage in a laboratory, and the questions of focus were: (1) Can unrewarding flowers function as landmarks? (2) How do bumblebees encode an array of artificial flowers in a laboratory setting? Specifically, do they encode the absolute or relative position of the rewarding flower, and do they base their choice on egocentric or allocentric information? (3) Do scent marks influence flower selection? This thesis does not therefore present a model of natural foraging behaviour, but instead concentrates on details of bumblebee memory that can be gained through artificial means. For instance, when faced with an unnatural situation such as a change in flowers' locations, what can be revealed about the content of bumblebee memory and the adaptability of bumblebee cognition?

Reference versus working memory. Whether referring to human or nonhuman animals, the concept of memory is often divided into different categories or processes, such as reference memory and working memory. The distinction between reference and working memory is often seen in research with nonhuman animals (Shettleworth, 1998).

Reference memory refers to information that is more permanently stored; it is "memory for the unchanging characteristics of a task" (Honig, 1978, as cited in Shettleworth, 1998, p. 237). The concept of working memory is regarded differently for humans and nonhumans, although the distinction is ambiguous (Baddeley, 1995). Honig (1978, as cited in Shettleworth, 1998) regards nonhuman working memory to be memory for a specific event or events on a particular trial and this memory can be reset when the animal embarks on a new trial.

The distinction between bumblebee reference and working memory is not a critical priority of this thesis. One could argue that the methodology used, i.e., presenting the artificial flowers in the same positions in space during training, taps into a different sort of memory compared to a methodology that involves, for example, presenting flowers in different positions each time a bee enters the flight cage to forage during training. The reason behind the method used here is that it is an externally valid procedure: in nature flowers maintain the same positions in space across time. Of interest are the contents of the bees' memory: what do they encode that enables them to select the flower that offers reward? Whether they use reference memory, working memory, or some other form of memory when making their flower choice can be debated. Granted, the contents of memory may differ between different types of memory, however the types of memory may be distinguished. In any event, this thesis is concerned with the contents of bees' spatial memory accumulated over the course of each experiment.

Methodological Issues

Subject self-selection. One idiosyncrasy of bumblebees pertinent to this research is that ultimately the experimenter cannot control which bees will forage at any given time. That is, workers cannot be forced to forage. I have attempted to stimulate foraging in my earlier research before my thesis work: When no bees were foraging I took bees from within the colony and placed them either in the corridor leading to the flight cage, in the flight cage, or on the rewarding flower. These attempts were unsuccessful in that the bees simply drank the sugar or honey solution, remained stationary in the cage, or flew back to the colony and stayed there. This occurred even when I selected bees that usually foraged regularly. I realized that the bees, and not the researcher, have control over who will be trained and tested, and when.

At any one time roughly one third of the workers of a colony are foragers (Alford, 1975); the other two-thirds remain in the nest to incubate and feed the larvae, defend against predators, and assist in thermoregulation of the nest (Heinrich, 1979a). Unlike honeybee workers whose roles are age-dependent, the age at which bumblebees begin foraging is variable: most newly-emerged workers tend to nest duties and then at some later point switch to foraging (Goulson, 2003). What controls the "on-off foraging switch" of a bee? Although this question has not been clearly answered, several influential factors have been identified. For instance, the energy state of the colony generally determines how many workers will forage at a time, with low nectar reserves leading to an increase in the number of bees who will forage (Cartar, 1992; Free, 1955). However, there are individual differences in terms of the threshold to which bees will respond to the colony resource levels (Cartar, 1992), and some workers never forage (Goulson, 2003; personal observation). Also, if a number of foragers are removed from a colony some "housekeeping bees" will switch to foraging. Conversely, if housekeeping bees are removed some foragers will revert back to within-nest duties (Free, 1955). Such task switching is a unique characteristic of bumblebee colonies (Goulson, 2003)—some workers have been observed switching back and forth between housekeeping and foraging several times within one day (Heinrich, 1979a; personal observation).

Although the exact mechanism that controls a bee's foraging behaviour remains a mystery, future foragers can sometimes be predicted based on size. Larger workers tend to forage more regularly and efficiently, while smaller workers tend to remain in the nest (Free, 1955; Goulson, 2003; Goulson, Peat, Stout, Tucker, Darvill, Derwent, & Hughes, 2002; Spaethe & Weidenmüller, 2002). It is possible that large body size permits foraging in lower temperatures and harsher weather (Free & Butler, 1959; Goulson et al., 2002). Less efficient foragers are often task-switchers who only forage when the colony energy level reaches a particular threshold, thus they do not forage on a regular basis (Cartar, 1992; Goulson, 2003).

All of this is to say that the best a researcher can do at present to recruit bumblebee subjects is to deprive the colony of nectar (so at least its surface nectar pots are empty) in order to stimulate foraging. Based on the findings above these bees will likely be the largest and most efficient workers. However, nectar deprivation beyond some (unknown) level increases the chance that less efficient foragers will begin to forage. Thus, the implications for this thesis are predicted as follows. First, each "cohort" of eggs the queen produces results in workers of varying sizes (Heinrich, 1979a). Inducing nectar deprivation throughout the experiment will result in the larger, more efficient workers of each cohort to forage. A number of the smaller, less efficient bees of each cohort may be stimulated to forage if the nectar deprivation level reaches a certain (unknown) point and/or when the larger, efficient bees are removed from the colony after they have been trained and tested. This resultant sampling of bees of varying foraging efficiencies will likely have implications for training and testing. For example, some foragers may require more training to learn how to obtain nectar from the artificial flowers. Given this, and also the fact that training criteria vary from study to study, often with no rationale (e.g., Burmeister et al., 1995), the number of flower visits during training will be included as a covariate. Incidentally, inducing nectar deprivation in the experiments of this thesis will allow for sampling of a wider distribution of workers within a colony (i.e., both efficient and less efficient workers), increasing the generalizability of the results beyond the most efficient foragers. It also mimics nectar deprivation that can occur in nature (e.g., due to inability to forage in harsh weather, removal of plant life by humans, etc.) as well as death or disappearance of "expert" workers that occurs in wild colonies (Heinrich, 1979a).

Elusive nature of scent marks. At present there is no way for humans to directly observe whether or not a bee has placed a scent mark on a flower. Moreover, scent marking behaviour is ultimately under the control of the bees. In other words, in this thesis scent marking is treated as an independent variable but ultimately it is a dependent variable. This problem is akin to that faced by researchers of mental imagery: although the experimenter can set the conditions for the phenomenon to occur, there is no guarantee that the subjects actually did conjure up an image / deposit a scent mark, and presently there is no way to unequivocally qualify or quantify it (Luce, 1979). The purpose of this thesis, however, is to study the possible interaction between spatial memory and what previous literature has termed scent marking. That is, here scent marking is treated as a possible memory mechanism. Therefore, although direct observation, measurement, and manipulation of scent marks would be ideal, for consistency with previous work and for lack of better technology, this thesis will treat this variable similar to how it has been treated in the past: allowing bees the opportunity to scent mark and observing their subsequent behaviour with the presumably marked flower cover. Conditions in which scent marks are allowed are termed conditions with "old covers," and conditions where possible scent marks are eliminated are termed conditions with "new covers."

Overview of Experiments

Chapter Two: Experiments 1 and 2. The first experiment was a pilot study with three purposes. First, it was designed to test whether bumblebees can discriminate between visibly identical flowers, specifically whether they can learn the position of a rewarding flower. Second, it tested Cameron's (1978, 1981) hypothesis that scent marks function as a "probe-or-not-to-probe" cue. Finally, it was an opportunity to fine-tune the apparatus and procedure for the remaining experiments of this thesis.

During training bumblebees were presented a row of three identical artificial flowers in a flight cage, one of the flowers offering honey solution (S+) and the others offering nothing (S-). Before being tested each bee was given the opportunity to scent mark the S+ by visiting the flower once and probing for nectar. For the test the flower covers were switched, replaced with completely unused covers, or left unchanged. The bee was released and her first flower choice was recorded either as an approach, a landing, or a probe. It was hypothesized that if bumblebees use spatial memory to select flowers, in testing they should choose the flower that is in the same position as the S+ in training regardless of whether or not it had an old or new cover. On the other hand, if scent marking is used to select flowers, then during testing bees would choose the flower that had the old, visited cover regardless of whether it had been moved to a new location in the row of flowers. For the test condition with new covers, if only scent marks are used for flower choice then bees should show no significant preference for any flower. Finally, if Cameron's hypothesis is correct, then bees should probe their flower of choice only if it has an old, visited cover; otherwise, they should show an approach or land response.

The second experiment used a similar procedure but during testing the S- were moved such that the S+ had a different position relative to the S-. To investigate the role of scent marking, flower covers remained unchanged for half of the bees, but for the rest of the subjects flower covers were replaced with new ones. The results indicate to what extent old, presumably scent marked covers influence flower choice, and whether bees base their test choice on memory for absolute position of the S+ or memory for relative position of the S+. If relative position is used this would suggest the S- functioned as landmarks.

Chapter 3: Experiments 3 and 4. These experiments are presented as part of a manuscript to be submitted for publication. Experiment 3 is a replication of Experiments 1 and 2 with the added condition of old, visited covers changing absolute position during the test, where the new position was outside of the original training array. This condition would indicate whether old, presumably scent marked covers play a role in flower choice when the flower array appears different from that in training, and the old, visited cover is no longer in the absolute training position of the S+. Finally, the purpose of Experiment 4 is to determine whether bumblebees remember the absolute position of the S+ using memory for a flight vector or flower array independent (FAI) information.

CHAPTER TWO

Experiment 1 (Pilot Study)

Method

Subjects

Seventy-two worker bees from three bumblebee colonies were used (24 workers from each colony). One colony of *Bombus impatiens* was obtained from Koppert Biological Systems, Inc. (Ann Arbor, Michigan). Two colonies of *Bombus bimaculatus* were reared in the lab from captured queens according to the procedure developed by Plowright and Jay (1966). Worker bees were labeled using coloured, numbered, plastic discs glued to the bees' thorax, or coloured paint applied to the bees' thorax. Pollen was provided to the colonies *ad libitum*.

Flight Cage and Housing

Figure 1 illustrates the arrangement of the colony and flight cage. Each colony was housed in a wooden container (30 cm x 15 cm x 15 cm) connected to a wooden corridor (30 cm long), which was in turn connected to a wooden framed, screened flight cage (183 cm x 188 cm x 190.5 cm). The corridor was covered with removable glass plates so that the experimenter could observe which bees were entering and exiting the flight cage. Two vertical plastic gates were inserted between the glass plates of the corridor so that the experimenter could control which bees entered and exited the colony and flight cage. Inside the flight cage radiating black stripes were placed around the hole to the corridor to aid the bees in returning to the colony. The floor of the flight cage was painted green and contained evenly spaced holes in which artificial flowers could be inserted (see below). Illumination was provided by fluorescent lights on the ceiling of the room as well as above the ceiling of the flight cage. The average temperature of the room was 20°C (range: 18°C-22°C).

Artificial Flowers

The artificial flowers were based on those used by Hartling and Plowright (1979;

see Figure 2). Their construction was such that the nectar level is not visible and they could be quickly refilled with nectar while the experimenter remained outside of the flight cage. Each flower consisted of a wooden box (12.5 cm long x 8 cm large) supported on a rod (60 cm high). The wooden box was covered by a purple, thin piece of plastic (duraflex) that could be easily removed and replaced. This is referred to as the flower cover.

Inside the box was a wooden platform that held a small plastic vial cap (2.5 cm diameter, approximately 5 ml) filled with honey solution (2 parts commercial honey to 1 part water by volume). Beside the wooden platform was an electromagnet attached to a small Teflon plate. The Teflon plate contained a capillary tube approximately 7.6 mm in length, situated such that it was directly above the vial cap. When the flower is not activated the Teflon plate rests up against the underside of the flower cover. The flower cover had a small hole such that when the cover is in place on the wooden box one can see the top of the capillary tube. Around the hole was a yellow ring to aid the bees in finding this nectar hole. The Teflon plate with the capillary tube could be removed for cleaning purposes.

To fill the flower with nectar one presses the flower's corresponding button on a 12-V battery-powered switchboard located outside of the flight cage. Pressing the button activates the switch, and the electromagnet in the flower pulls down the metal bar that is attached to the Teflon plate. This lowers the Teflon plate so the capillary tube is submerged in the honey solution in the vial cap. Surface tension allows the capillary tube to fill with nectar (*M* = 2.69 µL, *SE* = 0.19 µL; Hartling & Plowright, 1979). When the button is released (i.e., the electromagnet is deactivated), the Teflon plate returns to its original position up against the underside of the plastic cover, the hole of the plastic cover lined up with the top of the capillary tube. By probing the hole in the plastic cover bees thus probe the capillary tube and obtain a small amount of nectar. The amount of nectar in the capillary tube can be depleted by one bee visit. The capillary tube is refilled each time the flower's button is pressed. The amount of time needed to press the button and refill the capillary tube is approximately one second (Hartling & Plowright, 1979).

Three artificial flowers were used. They were placed in the flight cage side-by-side, 34 cm apart, such that when the bees first entered the flight cage they encountered a horizontal row of three identical flowers. The closest flower was 65 cm from the entrance to the corridor and the furthest flower was 105 cm from the entrance to the corridor (on a diagonal).

Procedure

Each day of the experiment proceeded as follows: (1) first step in training (free-foraging session), in which foragers were identified; (2) second step in training, in which bees were trained to find the nectar hole of the flower; (3) pre-test, in which bees were allowed to scent mark the rewarding flower; (4) testing, in which bees were exposed to one of the experimental conditions.

Training. Initially the bees had to learn to forage from the artificial flowers. Ideally each colony should have been exposed to all three rewarding positions, but because the first step in training consisted of free foraging, it was not possible to train different bees from each colony to different reward positions. Thus, each colony was randomly assigned a different rewarding flower (S+): for the first colony, S+ was the flower on the left; for the second colony, S+ was the middle flower; for the third colony, S+ was the flower on the right.

For the first step (free-foraging), three to five vial caps filled with honey solution (2 parts commercial honey to 1 part water by volume) were placed on top of the S+. This allowed the bees to find the reward using olfactory cues, as bees are attracted to the scent of honey. In order to facilitate foraging, colonies were deprived of nectar one to two days before training so that all surface honey pots in the colony were empty. Once the bees were released into the flight cage the experimenter recorded the identity of foraging bees. These were bees that repeatedly collected honey solution from the S+ and returned to the colony. Once one or more foragers were identified this training step ended and bees still in the flight cage were returned to the colony. This training step occurred each day for each colony to identify new foragers.

The second step was to train the identified foragers to probe the nectar hole of the S+. Several drops of honey solution were placed around the nectar hole of the S+ and its corresponding button on the switchboard was pressed, filling the capillary tube with honey solution. Bees previously identified as foragers were released into the flight cage. Once a bee landed on the S+ the experimenter observed the bee as she depleted the drops of honey solution. After the drops were depleted bees often swiped their proboscis across the surface of the flower; the experimenter observed whether during this process the bee found the nectar hole. When a bee found and subsequently probed the nectar hole it was readily observable: her proboscis visibly went down into the hole and the bee remained stationary for several seconds while she imbibed the honey solution. The capillary tube of the S+ was filled for each bee visit, thus giving each bee a reward on each visit to the S+. The number of times each forager probed the hole was recorded. This training period stopped after one or more foragers were observed consistently probing the S+.

Pre-test and Testing. Each forager who was identified as probing the S+ during training was subsequently exposed to the pre-test and test. Figure 3 illustrates training and testing for each condition.

For each forager in the pre-test, all flower covers were replaced with new, unused covers to eliminate all possible odours from the honey solution and foraging bees. The experimenter pressed the S+'s button on the switchboard, filling the flower's capillary tube with honey solution. The inner vial cap of the rewarding flower was removed, thus the forager could only receive a reward on her first probe and there would be no possible scent of honey solution emanating from within the S+. A forager who was observed consistently probing the S+ during training was released into the flight cage. She was allowed to probe the S+ and extract all of the honey solution in the capillary tube. Once she had drained the capillary tube and left the S+, the pre-test ended. At this point the only possible odour or residue on the flower cover would be the individual forager's ostensible scent mark left after probing.

Immediately after the forager probed and alighted from the S+, testing began. The experimenter turned off all lights in the testing room except for one red light. This was done to limit the bee's flight in the cage as the experimenter entered the cage to manipulate the flowers. Flowers were manipulated according to one of three test conditions (see Figure 3): (1) Old Covers Not Moved: flower covers and flower positions were unchanged; (2) Old Covers Moved: the flower cover of the S+ was switched with a flower cover of one of the S-; or (3) New Covers: all flower covers were replaced with entirely new, unused flower covers. For each condition the experimenter also replaced the Teflon plate and capillary tube of the S+ with a Teflon plate and capillary tube never exposed to honey solution. This eliminated choice of flower based on any scent cues detected in the capillary tube of the S+.

Once the experimenter had manipulated the flowers and exited the flight cage, the lights were turned back on and the red light was turned off. (The time required to turn off lights, manipulate the flowers, and turn lights back on was between one to two minutes.) Once the lights were back on, the forager in the cage resumed flight and the experimenter recorded her first flower choice as an approach, land, or probe. An approach was defined as flying 2 cm or less above the nectar hole and turning away without landing. The landing response was contact of the bee's feet on the flower and the bee did not probe the hole. Finally, the probe response required landing on a flower and probing its nectar hole (Schmitt & Bertsch, 1990). After the bee's first response she was captured and not used for the remainder of the experiment.

Twenty-four foragers from each of the three colonies were used (therefore 72 bees for the entire experiment). Each bee was randomly assigned to one of the three test conditions. For the second condition in which the flower cover of the S+ was switched with the flower cover of one of the S-, the S+ was switched with each of the two S- an equal number of times for each colony.

Results

Two analyses were conducted. The first was a χ^2 test for goodness of fit to compare the frequency of choice by bumblebees for the position of the S+ in training to chance (i.e., .33). Choice frequencies are displayed in Table 1. The second was a logistic analysis for binomial proportions using GLIM version 4.0 statistical software (Francis, Green, & Payne, 1993). This investigated the influence of colony and assignment of S+ in training on the choice by bumblebees for the training position of the S+. The factor of interest, nature of the testing flowers (scent mark not moved, scent mark moved, or absence of scent mark), was entered last into the linear model. For these analyses choice by bumblebees for the training position of the S+ was assigned a value of 1, whereas choice of other position was assigned a value of 0. Results of GLIM analyses are presented in χ^2 values, but these are not to be confused with the χ^2 values for the test of goodness of fit in the first analysis. Frequency of probing behaviour was investigated in a similar manner: probe responses were assigned a value of 1, approaches or landings a value of 0, and the factor of interest, nature of the testing flowers (scent mark not moved, scent mark moved, or absence of scent mark) was entered last into the model. Table 1 contains response type frequencies for each test condition. Individual data appears in Appendix A, and Appendix C provides a sample GLIM output from Experiment 2 of Chapter Two (chosen for its smaller data set).

Comparisons with a Theoretical Value of Chance

Condition 1: Old Covers Not Moved. For this condition choice of flower could have been made using memory for position of S+, presumed presence of scent mark, or both, as flowers were not manipulated. As Table 1 illustrates, bees chose the flower that was in the same position as the S+ during training at a frequency significantly above chance: $\chi^2(2, N = 24) = 31.75, p < .05$.

Condition 2: Old Covers Moved. When the probed flower cover was switched with one of the other two covers, bees responded as though they were choosing flowers based memory for the position of the S+ and not presence of scent mark. They selected the flower that was in the original position of the S+ significantly more often than the other two flower positions: $\chi^2(2, N = 24) = 19.00, p < .05$ (see Table 1).

Condition 3: New Covers. Replacing all of the flower covers with new, unused covers resulted in a significant number of responses that would be expected if bees were using memory for the original position of the S+. As seen in Table 1, the number of choices for the flower in the original position of the S+ was greater than would be expected by chance: $\chi^2(2, N = 24) = 19.75, p < .05$.

Effects of Experimental Variables

Flower choice. Colony (χ^2 $(2, N = 72) = 3.57, p > .05$) and position of S+ in training ($\chi^2(1, N = 72) = 0.56, p > .05$) did not have a significant influence on flower choice during testing. If choice is considered a binary variable where 1 = choice of original position of S+ and 0 = choice of other position, the choice means show that the flower in the same position as the S+ was chosen most often when there was an old, unmoved flower cover (Condition 1: $M = 0.88$, $SD = 0.34$) compared to an old, moved cover or new cover (Condition 2: $M = 0.75$, $SD = 0.43$; Condition 3: $M = 0.75$, $SD = 0.43$). However, this difference was not significant ($\chi^2(2, N = 72) = 1.75, p > .05$). There were no significant interactions between nature of testing flowers and position of S+ in training ($\chi^2(2, N = 72) = 1.26, p > .05$), and nature of testing flowers and colony ($\chi^2(2, N = 72) = 1.68, p > .05$).

Response type. Probing occurred significantly more often when flowers had old covers compared to when flowers had new covers: $\chi^2(2, N = 72) = 36.39, p < .05$; see Table 1.

Discussion

Bumblebees in this experiment learned the position of the rewarding flower presented to them in training. In Condition 1: Old Covers Not Moved, bees showed a significant preference for the flower in the same position as the S+, and they could use spatial memory, scent marks, or both to make this decision. Bees again chose the flower in the S+ position above chance levels in Condition 2: Old Covers Moved, but here the flower in this position had no scent mark, suggesting spatial memory was behind their choice. Condition 3: New Covers is especially convincing that spatial memory and not scent marks was responsible for bees' flower selections because all possible scent marks were completely eliminated, yet bees still showed a significant preference for the flower in the S+ position. If scent marks played a role in flower choice, then in Condition 2: Old Covers Moved, bees should have chosen the flower that had the presumably scent marked cover regardless of its position in the row of flowers. Moreover, in Condition 3: New Covers, bees should have shown no significant flower preference. Thus, to locate the S+ bees seem to remember its position in space rather than relying on a physical cue they may have placed upon it.

The results here also support Cameron's (1978, 1981) theory that scent marks function as a "probe-or-not-to-probe" cue. Probing behaviour occurred above chance levels only when there was opportunity for a scent mark on the chosen flower. If there was no opportunity for a scent mark bees tended instead to land or approach their selected flower. Therefore, scent marks did not influence flower selection but their presence or absence appeared to control behaviour once the bee was on or near the flower.

There are two possible reasons why there was more of an effect of memory for flower position compared to the experiments on scent marking by Cameron (1978, 1981) and Schmitt and Bertsch (1990). First, in the present study only the first flower choice for each bee was considered, whereas Cameron and Schmitt and Bertsch used frequency of visits to each flower as their dependent variable. The order in which flowers were visited in these two studies was not reported. Second, this study included a test condition in which bees were forced to choose from flowers that had absolutely no possible traces of scent marks (Condition 3: New Covers). Cameron's (1978, 1981) study did not include such a condition, whereas Schmitt and Bertsch (1990) cleaned rewarding flowers with pentane and hot water in an attempt to eliminate scent marks. Bees in Schmitt and Bertsch's experiments, when encountering cleaned, hopefully scent-mark-free flowers, "became confused" and began to probe the S-. No such behaviour was observed in this experiment. When bees encountered new, unused flower covers they first tended to select the flower in the position of the S+ but did not probe the flower. Bees continued to land several times on this flower before landing on any of the other flowers, or flew around the flight cage after their first response. Thus, although our bees responded differently to scent-mark-free flowers compared to bees observed by Schmitt and Bertsch, it appeared that, as Schmitt and Bertsch have suggested, foraging efficiency suffers without scent marks.

The main conclusion from this experiment is that bumblebees remembered the position of the S+ flower that was situated amongst two unrewarding flowers. How did they accomplish this? That is, what was the nature of the bees' spatial memory? Did they remember the absolute position of the rewarding flower in the flight cage regardless of the positions of the S-, or did they encode the rewarding flower's position relative to the positions of the unrewarding flowers? Specifically, did the S- function as landmarks? Experiment 2 addresses these questions.

Experiment 2

As in the previous experiment, bees were presented a row of three artificial flowers, one offering reward. Once bees had learned to probe the S+ for nectar they were presented a test where the two S- were moved so that the flower in the S+ position remained in the same absolute position but was now in a different position relative to the other two flowers (see Figure 4). If bees use memory for the absolute position of the S+, then they should choose the test flower that is in the same position as the S+ in training even though the S-'s are in different locations. On the other hand, if memory for the relative position of the S+ is used, then bees should choose the test flower that is in the same position relative to the other flowers. In this case the positions of the S- influence choice, indicating they were used as landmarks.

As in Experiment 1, Experiment 2 includes a test condition where all flower covers are replaced, eliminating any influence of scent marks. This will be compared to a condition where flower covers are not manipulated, ergo allowing the presence and possible influence of scent marking. Based on the results of Experiment 1 and the research by Cameron (1978, 1981), in Experiment 2 bees should only probe their flower of choice if the cover was allowed to have a scent mark.

Considering Experiment 1 was a pilot study to refine the apparatus and procedure for the remaining experiments for this thesis, several necessary improvements were implemented in Experiment 2 (carried over into Experiments 3 and 4). First, some alterations were made regarding the artificial flowers. It is possible that after bees probed the S+ during the pre-test stage in Experiment 1, traces of honey solution were left on the flower cover. Thus, the differences in approaching, landing, and probing frequency may have been due to the presence or absence of the scent of honey rather than scent marks. In Experiment 2 sugar solution was used instead since bees cannot detect it by odour (Brown & Demas, 1994; Couvillon & Bitterman, 1991; Giurfa et al., 1994). The only possible olfactory cue that the bees could use in Experiment 2 is scent marks.

Next, flower covers in Experiment 2 consisted of yellow duraflex instead of purple. Yellow, a colour bees can easily detect (Dukas & Real, 1993; Free, 1970), contrasted more with the dark green floor of the flight cage compared to the purple covers. Yellow flowers would thus be more prominent and easier for the bees to detect in the cage. In addition, during Experiment 1 it was observed that bees had difficulty locating the nectar hole, possibly because bees tend not to show a strong preference for concentric patterns (Lehrer et al., 1995), i.e., the yellow ring placed around the nectar hole. Since bees tend to show greater preference for radial patterns (Lehrer et al., 1995) and radial patterns can act as floral nectar guides (Free, 1970), the nectar hole of the artificial flowers was encircled with a white ring of radiating blue stripes. Blue is a colour that is also easily perceived by bees (Dukas & Real, 1993; Free, 1970). All of these visual adjustments to the artificial flowers were made with the intention to aid bees in the training process of locating both the flowers and the source of nectar.

Subsequently, for safety reasons the location of the flower array within the cage was changed. In Experiment 1, during training when the vial caps or drops of honey solution on the S+ were depleted and foragers had not yet learned to probe the nectar hole, the experimenter had to enter the cage, which was full of bees, to replace the drops of honey solution. In Experiment 2 the flowers were placed in the flight cage side-by-side, 20 cm apart, such that when the bees entered the flight cage they encountered a row of flowers close to and parallel to the left-hand wall of the flight cage. This way the experimenter did not have to enter the cage to replenish the sugar solution: he/she simply had to stick the end of a syringe through the screen of the flight cage, aim it at the rewarding flower surface, and depress the syringe so that the sugar solution landed on the flower cover or in the vial cap. This method also saved time: the experimenter did not have to capture bees that escaped from the flight cage and place them back into the cage or colony.

Finally, one variable that was not measured in Experiment 1 was the number of rewards obtained by each bee during training. This aspect of the bees' learning history could influence their test choice and perhaps their scent marking behaviour and/or scent mark detection. Thus, for Experiment 2 both the number of rewarding and unrewarding visits to the flowers during training were recorded for each bee and entered as covariates in the logistic analysis.

Method

All aspects of the methods were the same as in Experiment 1, except for differences described in the Discussion above, and for the following.

Subjects

Twenty-four worker bees from two new colonies of *Bombus impatiens* were used (12 from colony 1 and 12 from colony 2). Both colonies were obtained from Koppert Biological Systems, Inc. (Ann Arbor, Michigan).

Procedure

Training and testing procedures for all conditions are displayed in Figure 4.

Training. Honey solution was used only at the very beginning of training to allow bees to find the rewarding flower using scent; it was gradually replaced with sugar solution, which has no scent. For the bees' first exposure to the flowers, the S+ contained one vial cap of honey solution and the other vial caps were filled with sugar solution. The switchboard button corresponding to the S+ was pressed, filling its capillary tube with sugar solution. Once bees had foraged from the vial caps they were removed and replaced with drops: one small drop of honey solution was placed on the flower cover and several large drops of sugar solution were placed closer to the nectar hole. Once the drop of honey solution was depleted it was not replenished but sugar solution drops were always replenished. When bees began to consume the drops of sugar solution after the honey solution drop was consumed, the flower cover of the S+ was replaced with a new cover so that there would be absolutely no trace of honey scent on the S+. Bees now had to locate the S+ without using any olfactory cues from the sugar solution. Drops of sugar solution were placed around the nectar hole. The experimenter observed which bees found and probed the nectar hole as in Experiment 1. Bees were tested if, after complete removal of honey solution, they flew to the S+ consistently without landing on or approaching the S-, and if they probed the nectar hole of the S+ more than once.

Pre-test and Testing. Testing involved moving one of the two S- such that the S+ maintained its absolute position in the cage but its position relative to the S- was different from that in training. For each of the three possible training positions of the S+ (outer left, middle, or outer right), there were two test manipulations corresponding to the two possible changes in relative position. For example, if in training the S+ was located in the middle, there were two possible test situations: move the S- on the outer left-hand side to the outer right-hand side of the array, resulting in the S+ being the outer left-hand flower; or, move the S- on the outer right-hand-side to the outer left-hand side, resulting in the S+ being the outer right-hand flower (see Figure 4).

In regards to the manipulation of scent marks presumably present on flower covers, in Condition 1: Old Covers, the flower covers were not manipulated during testing (see Figure 4), allowing bees to respond to scent marks. In Condition 2: New Covers, all flower covers were replaced with new, unused covers to eliminate any possible scent marks (see Figure 4).

As explained in Experiment 1, the nature of free foraging did not allow bees from each colony to experience all possible positions of the S+ during training. Instead, colony #1 was randomly assigned the S+ in position one. Except for four bees from colony #1 who provided data for S+ in position two, the declining health of colony #1 resulted in colony #2 providing data for S+ in positions two and three.

Results

Appendix A displays individual data. A χ^2 test for goodness of fit was performed on the data for each condition to determine whether during testing foragers chose the flower that was in the same absolute position as the S+, or a flower in another position, above chance levels (i.e., .33). Table 2 displays frequencies for flower choice and χ^2 values for each condition.

A logistic analysis using GLIM was performed in order to investigate the influence of colony, proportion of rewarding visits in training (number of rewarding visits in training / total number of flower visits in training), and assignment of S+ in training, on the choice of absolute position of the S+. Presence or absence of scent mark was entered last in the analysis. GLIM was also used to compare the frequency of probing behaviour to the combined frequencies of all response types. Data for response type can be found in Table 2.

Comparisons with a Theoretical Value of Chance

Condition 1: Old Covers. As shown in Table 2, in this condition where bees had the opportunity to use scent marks, they chose the flower that was in the same absolute position as the S+ significantly more frequently than chance: $\chi^2(2, N = 12) = 14.00, p < .05$.

Condition 2: New Covers. When flower covers were replaced with new, unused flower covers during testing, bees no longer showed any significant flower preference: $\chi^2(2, N = 12) = 2.00, p > .05$ (see Table 2). However, choices were in the direction towards absolute position of the S+.

Effects of Experimental Variables

Flower choice. Colony ($\chi^2(1, N = 24) = 3.10, p > .05$) and assignment of S+ in training ($\chi^2(2, N = 24) = 0.28, p > .05$) did not have any significant influence on flower choice during testing. However, there was a significant main effect of proportion of rewarding visits during training ($\chi^2(1, N = 24) = 6.76, p < .05$), with bees who choose the absolute position having a lower mean proportion of rewarding visits during training than bees who did not choose absolute position ($M = 0.62, SD = 0.19$ and $M = 0.74, SD = 0.16$, respectively). Presence or absence of scent marks also showed a significant main effect ($\chi^2(1, N = 24) = 10.18, p < .05$). If flower choice is considered as a binary variable, with 1 = choice of absolute position of S+ and 0 = choice of other flower position, the choice mean is higher for Condition 1: Old Covers ($M = 0.83$, $SD = 0.37$) than for Condition 2: New Covers ($M = 0.50$, $SD = 0.50$). Thus, bees were more likely to choose absolute position if there was a presumed scent mark on the flower. Finally, there was a significant interaction between number of rewarding visits in training and the allowance or absence of scent marks: $\chi^2(1, N = 24) = 7.32, p < .05$. Bees in Condition 1: Old Covers had a higher mean number of rewarding visits during training ($M = 33.25$, $SD = 21.79$) than bees in Condition 2: New Covers ($M = 22.17$, $SD = 21.02$). In sum, these results suggest the proportion and number of rewarding visits in training, and the absence or presumed presence of scent marks, influenced choice of absolute position.

Response type. When it was possible for test flowers to have scent marked covers (i.e., Condition 1: Scent Mark), probing occurred more often: $\chi^2(1, N = 24) = 16.73, p < .05$ (see also Table 2). Therefore, as in Experiment 1, scent marks influenced response type by acting as a "probe-or-not-to-probe" cue.

Discussion

In general bumblebees in this experiment selected test flowers based on memory for absolute position of the S+. Although this result was not significant for Condition 2: New Covers, absolute position still received the majority of choices in this condition (see Table 2). It is quite possible that relative position of the S+ was encoded but bees nevertheless did not use this information when making a choice. Since memory for relative position was not used, this suggests the unrewarding flowers did not function as landmarks for locating the S+. This is despite the fact that they were salient--bright yellow and contrasting with the dark green floor of the flight cage--and they were close to the S+. Salience and proximity, speculated to be important characteristics of landmarks (Cheng et al., 1986; Cheng, 2000; Chittka et al., 1995), were not sufficient in this case to result in bees using the S-'s as landmarks. Perhaps different results would be obtained with this paradigm if these two characteristics were more clearly defined. For instance, salience may require visual distinction from the S+. Here the S-'s were visually identical to the S+; rendering them a different colour from the S+ could make them more "salient" in their own right. Proximity is also a vague prerequisite, with no specific distance parameters specified. In this thesis the S- were not landmarks when they were each 34 cm away from the goal, yet Huber and colleagues (1994) found that a bar functioned as a landmark when it was placed only 5 cm from the goal. In sum, although the term "landmark" is a term frequently found in the literature, more investigation is required to clarify what exactly this term refers to within the context of bee foraging behaviour.

How the bees encoded the absolute position of the S+ is not clear from the results obtained here. Bees might have used some other object in the laboratory; however, what this object could have been is unknown. The experimenter herself would have been unreliable as a landmark as she did not remain in one position throughout the procedure. The encoding of absolute position will be investigated in greater detail later in the next series of experiments.

As in Experiment 1, ostensible scent marks functioned as a "probe-or-not-to-probe" cue: more probing behaviour appeared in Condition 1: Old Covers compared to Condition 2: New Covers. However, one curious result is that presumed presence of scent marks seemed to control choice for absolute position: absolute position was selected significantly above chance levels in Condition 1: Old Covers, but dropped to chance levels in Condition 2: New Covers. Therefore, scent marks may have played a role in flower selection, which is contrary to the finding in Experiment 1 that scent marks influenced behaviour *after* floral choice. Admittedly, it is difficult to generalize the results found here to those of Experiment 1 given the number of changes made to the artificial flowers. Experiment 3, found within the manuscript below, was designed to replicate Experiments 1 and 2 using similar apparatus.

Lastly, the two covariates proportion of rewarding visits in training and number of rewarding visits during training were each found to exert an influence on flower choice during testing. In general, within the range of observations in this thesis, bees were more likely to choose absolute position during testing if they experienced a lower proportion of rewarding visits during training. Also, in general, a higher number of rewards in training, in combination with a presumably scent marked cover, also resulted in choice of absolute position. It is possible that these covariates are significant in this experiment as a result of the relatively limited range of number of rewarding visits during training. The number of rewarding visits ranged from 2 to 81, whereas in Experiment 3 (Experiment 1 of the journal manuscript), the number of rewarding visits in training ranged from 5 to 147. As will be seen, with this larger range of rewarding visits in Experiment 3, the opposite finding was obtained: the higher the mean proportion of rewarding visits in training, the higher the choice frequency for FAI position (see page 72). For the purpose of this thesis a detailed analysis of these covariates to uncover relationships between training and testing will not be performed. It is emphasized, however, that the effects of interest were significant *after* the covariates were entered into the logistic analysis.

The influence of variability in training rewards on test behaviour is an important avenue for further study. This is especially true given the lack of a training criterion for bee research and the different criteria used between studies. To maintain consistency with the first two experiments in this thesis, rather than disentangle an appropriate training criterion for Experiments 3 and 4, proportion of rewarding visits and number of rewarding visits during training will continue to be entered as covariates into the analyses. Any significant influence on the results will be noted.

Returning to the three main questions of this thesis (i.e., Can unrewarding flowers function as landmarks? How do bumblebees encode the array of artificial flowers? Do scent marks influence flower selection?), their answers thus far are as follows. First, there is no evidence at this point that unrewarding flowers can function as landmarks in the laboratory setting we implemented. Bumblebees in Experiment 2 tended to choose the test flower that was in the same absolute position as the S+, not the flower that was in the same relative position as the S+. Second, as results from Experiment 2 suggest, bumblebees encoded the absolute position of the S+ but how this is done remains to be seen. Finally, Experiment 1 showed that scent marks influenced bees' behaviour *after* flower selection, functioning as a "probe-or-not-to-probe" cue. However, results from Experiment 2 propose that scent marks may have influenced flower choice; specifically, absolute position failed to be chosen at levels significantly above chance when scent marks were absent.

What follows are the last two experiments of this thesis, included as part of a manuscript submitted for publication. The first experiment (Experiment 3 of this thesis) is an amalgamation of Experiments 1 and 2 in order to replicate their results using uniform apparatus, considering that generalization between Experiments 1 and 2, as they stand, is difficult. Experiment 3 also explicitly manipulated the movement of flowers outside of the training array: another difference that exists between Experiments 1 and 2. Finally, Experiment 3 included the new test condition whereby the presumably scent marked flower cover was placed in a location outside of the original training array. If, as results of Experiment 2 suggest, bees select flowers based on presence of scent marks, then bees in Experiment 3 should choose the flower with the presumably scent marked cover even though this flower is in a position not present during training.

The second experiment of the manuscript (Experiment 4 of this thesis) was dependent upon the results of Experiment 3. That is, whether in Experiment 3 bees used memory for absolute position of the S+ or memory for relative position of the S+, Experiment 4 was intended to investigate further the nature of this remembered information. Specifically, if memory for absolute position is used, how exactly is absolute position encoded? Similarly, if relative position is used, how is it encoded?

Finally, worth noting is the second author of the journal manuscript is my Thesis Supervisor. Our individual contributions are as follows. My Supervisor created the design for Experiment 1, assisted me with the interpretation of the statistical analyses, and provided constructive criticism on the writing and organization of the manuscript. I conducted all of the research required for the literature review, designed Experiment 2, collected all data for both experiments, ran the statistical analyses, created all tables and figures, and did all of the writing (including revisions).

Table 1.

Observed Frequencies of Test Flower Choices, Expected Values According to Chance, and Response Types for Experiment 1

	S+ Position	Other	# Probes / Total Responses
	Condition 1: Old Cover Not Moved		
Observed*	21	3	22 / 24
Expected	8	16	
	Condition 2: Old Cover Moved		
Observed*	18	6	11 / 24
Expected	8	16	
	Condition 3: New Covers		
Observed*	18	6	3 / 24
Expected	8	16	

Note. For each condition $df = 2$ and $n = 24$.

*Observed frequencies significantly different from expected values at $p < .05$.

Table 2.

Observed Frequencies of Test Flower Choices, Expected Values According to Chance, and Response Types for Experiment 2

	Absolute Position	Relative Position	Other	# Probes / Total Responses
		Condition 1: Old Covers		
Observed*	10	2	0	12 / 12
Expected	4	4	4	
		Condition 2: New Covers		
Observed	6	4	2	3 / 12
Expected	4	4	4	

Note. For each condition $df = 2$ and $n = 12$.

*Observed frequencies significantly different from expected values at $p < .05$.

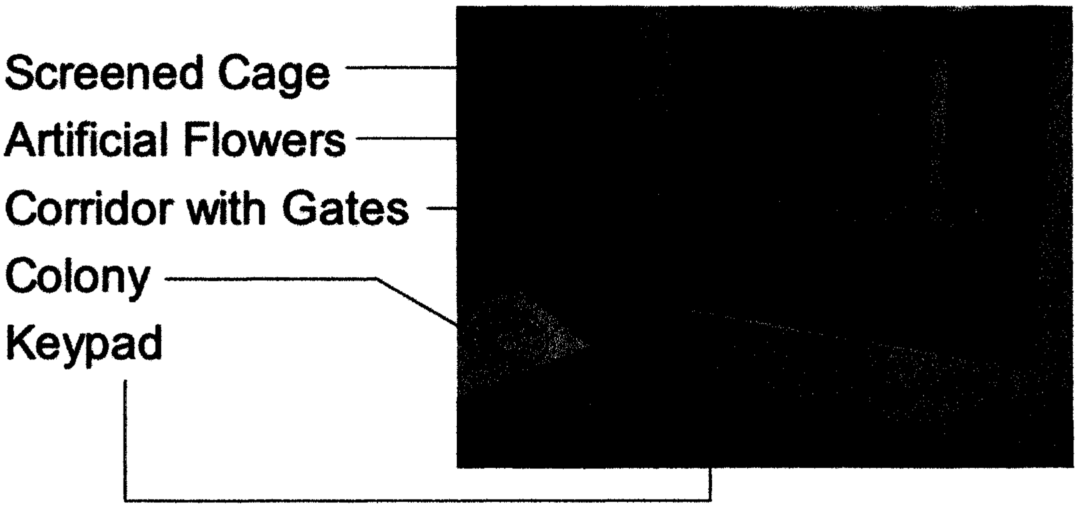
Figure Captions

Figure 1. Photograph of the apparatus used in all experiments. In Experiments 1 and 2 colonies were housed in a wooden container; in Experiments 3 and 4 colonies were housed in a plastic container within a styrofoam-insulated cardboard box provided by Biobest (shown here).

Figure 2. Bird's eye view (top) and cross section (bottom) of an artificial flower used in all experiments. Redrawn with permission from Hartling and Plowright (1979, p. 1867).

Figure 3. Illustration of training and testing procedures of all conditions in Experiment 1. S+ = rewarding flower (containing honey solution); S- = unrewarding flower (containing nothing). Circle on S+ = presumed scent-mark deposited after bee extracts nectar. Arrow indicates the position of the S+.

Figure 4. Illustration of training and testing procedures of all conditions in Experiment 2. S+ = rewarding flower (containing sugar solution); S- = unrewarding flower (containing nothing). Positions of the S+ and S- were counterbalanced across subjects. Circle on S+ = presumed scent-mark deposited after bee extracts nectar. Arrow indicates the absolute position of the S+.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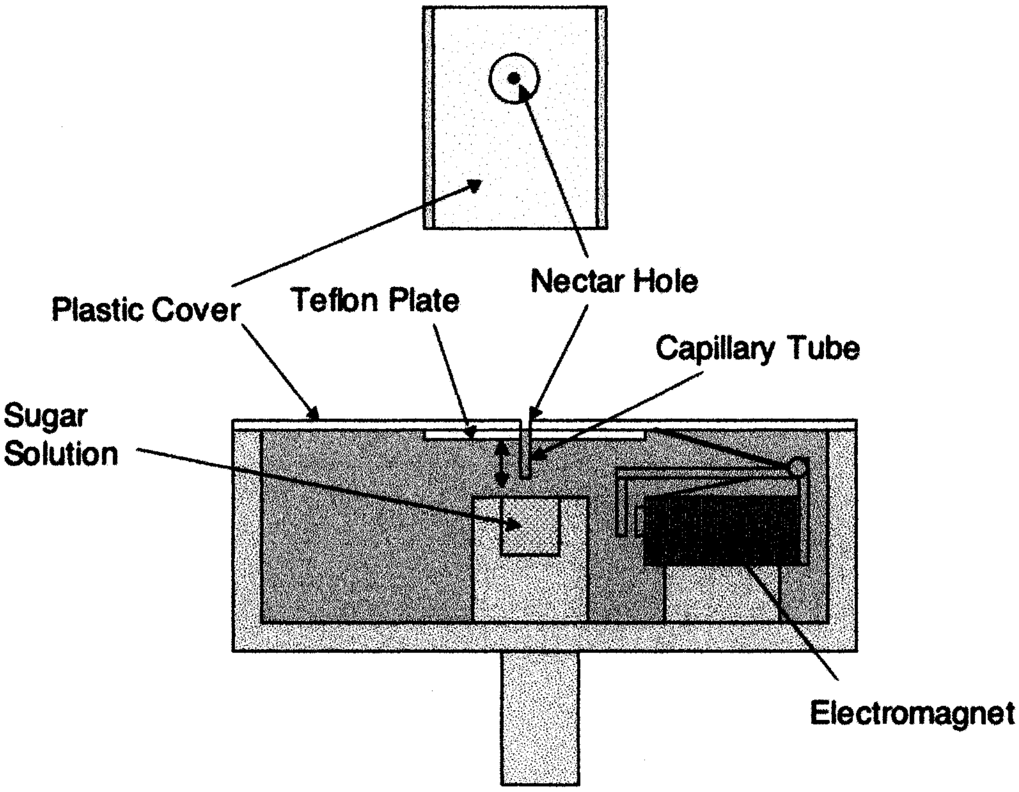
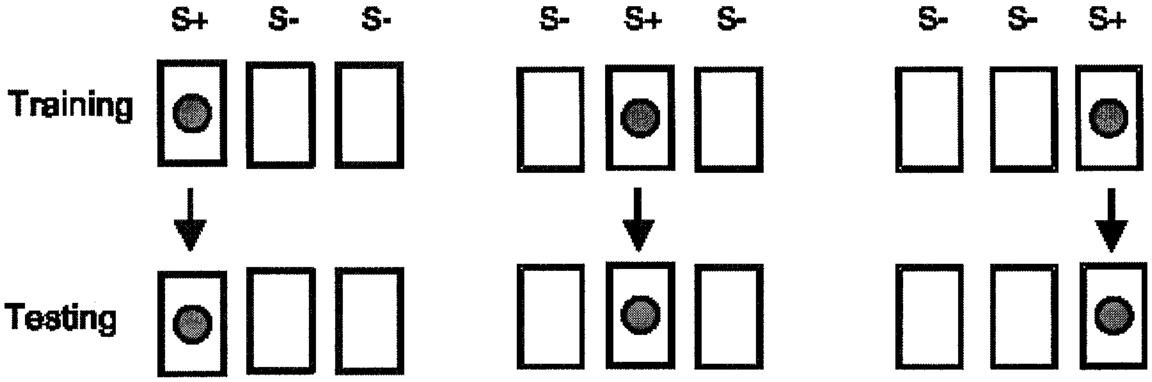
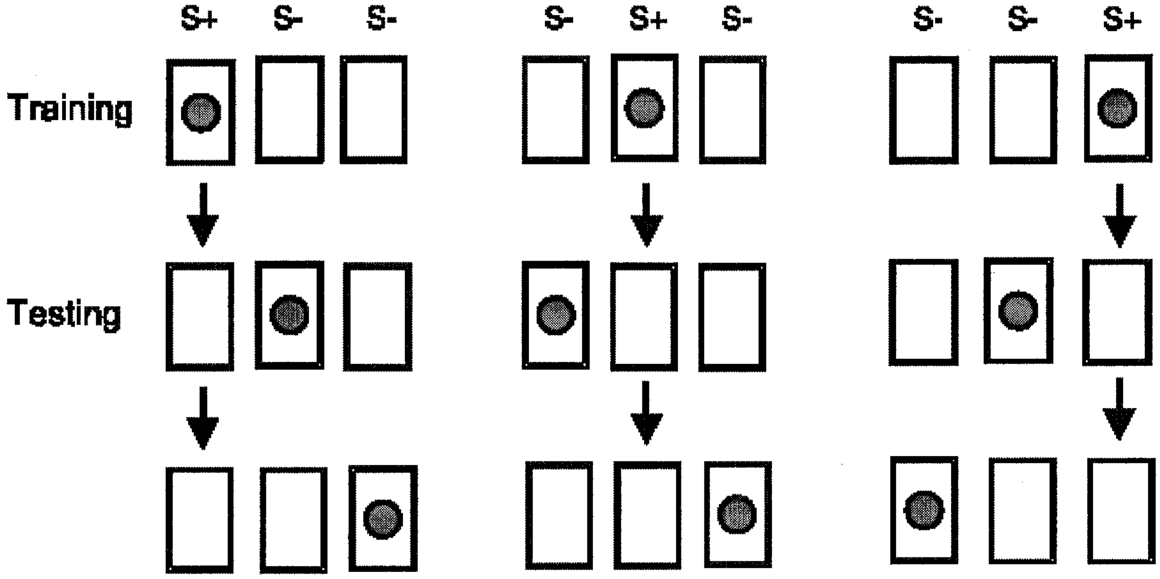
Artificial Flowers
Corridor with Gates
Colony
Keypad


Plastic Cover
Teflon Plate
Nectar Hole
Capillary Tube
Sugar Solution
Electromagnet

Condition 1: Old Covers Not Moved



Condition 2: Old Covers Moved



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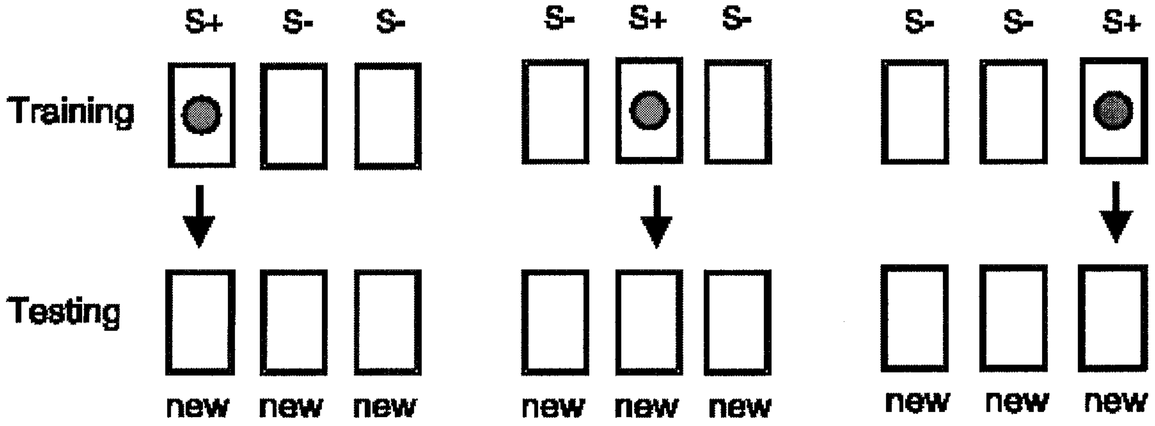
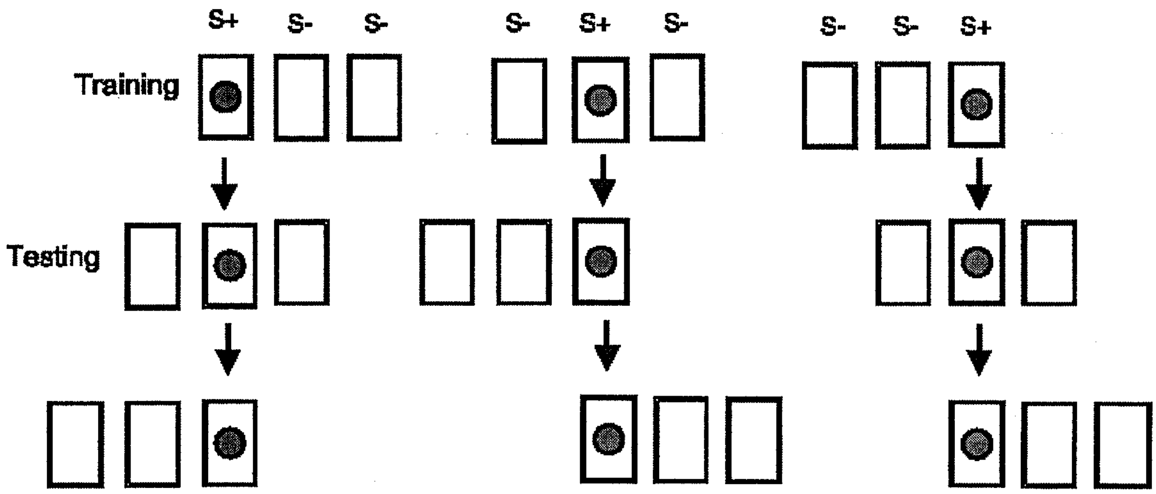
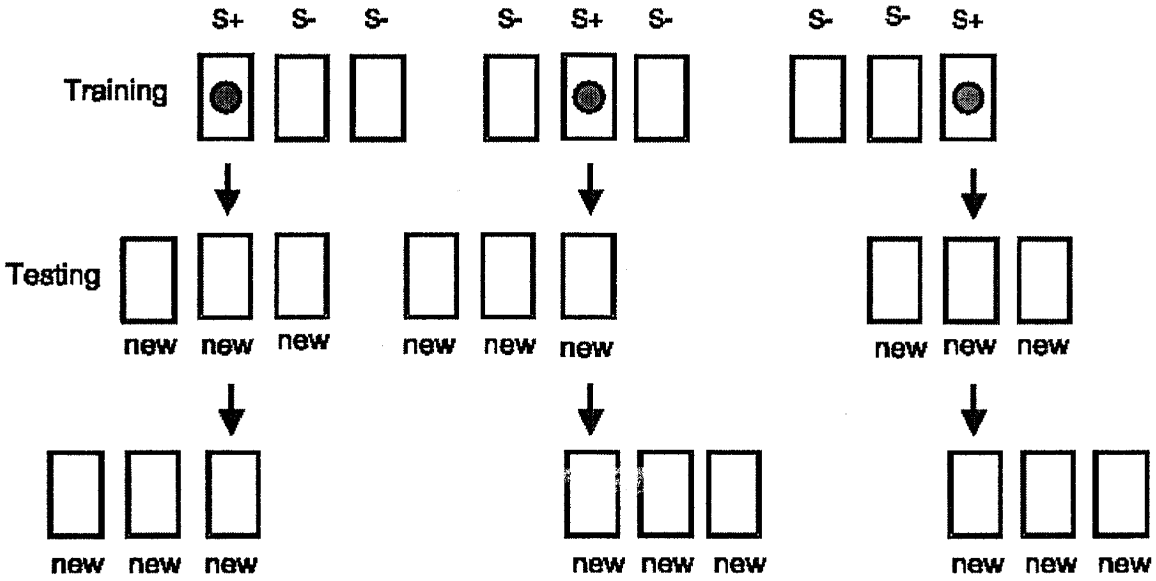


Condition 1: Old Covers



Condition 2: New Covers



Abstract

To determine what spatial information bumblebees (*Bombus impatiens*) use to encode the position of a rewarding flower, artificial flowers were placed in a flight cage, one offering reward (S+), the others empty (S-). Bees were tested with empty flowers. In Experiment 1, flowers were either moved at the time of testing or not. Bees returned to the flower in the same absolute position of the S+ (termed the flower-array-independent (FAI) position), even if it was in the wrong position relative to the S- and even when new flower covers prevented the use of possible scent marks. New flower covers (i.e., without possible scent marks) had the effect of lowering the frequency of probing behaviour. In Experiment 2, the colony was moved in between training and testing. Again, bees chose the flower in the FAI position of the S+, and not the flower that would be chosen using strictly memory for a flight vector. Together, these experiments show that to locate the S+ bees did not rely on the positions of the other flowers, though they were prominent objects close to the goal. Also, despite foraging in cue-impoverished surroundings, bees nevertheless selected environmental features to remember the position of the S+ instead of relying upon a purely egocentric point of view.

Keywords: bumblebees, spatial memory, egocentric information, landmarks

Spatial Encoding of Artificial Flowers by Bumblebees (*Bombus impatiens*): The Contents of Memory

Introduction

Bees are very good at locating a target in space. This applies to both large-scale, natural surroundings (Cheng, 2000; Collett, 1996; Dyer, 1998; Gould, 1984; Heinrich, 1976; Menzel, Gumbert, Kunze, Shmida, & Vorobyev, 1997; von Frisch, 1967), and smaller-scale, artificial environments (Brown & Demas, 1994; Brown, Moore, Brown, & Langheld, 1997; Cheng, 2000; Demas & Brown, 1995; Dukas & Real, 1993; Dyer, 1998; Giurfa, 1996; Gould, 1984; Greggers & Menzel, 1993; Heinrich, 1979; Isnec, Couvillon, & Bitterman, 1997; Ney-Nifle, Keasar, & Shmida, 2001). Their success has been attributed to tactics such as landmark use (Cartwright & Collett, 1982, 1983; Cheng, 2000; Chittka & Geiger, 1995a; Chittka & Geiger, 1995b; Chittka, Geiger, & Kunze, 1995; Collett, 1992; Collett, 1996; Collett, 1998; Collett & Collett, 2000; Couvillon & Bitterman, 1992; Dyer, Berry, & Richard, 1993), retinal image matching (Cheng, 2000; Cartwright & Collett, 1983; Collett, 1996; Collett & Cartwright, 1983; Gallistel, 1990), and path integration (Collett, 1996; Collett & Collett, 2000; Menzel, Geiger, Jeorges, Müller, & Chittka, 1998). Cheng (2000) has organized these processes into a theory postulating that bees follow a set of cognitive and sensorimotor steps when returning to a goal. As a result of this research, the process of *how* bees return to a place has become clearer. However, *what* spatial information the bee encodes about a goal remains to be elucidated. In this paper we extend previous work by investigating the specific contents of bee memory when the animal returns to a rewarding flower.

To determine what spatial information bees encode about a reward, we have pared the situation down to its bare essentials: a colony of bumblebees, a flight cage, and two to three artificial flowers. Artificial flowers were presented in a row, one offering reward (S+) while the others offering nothing (S-). The position of the S+ could be remembered in three ways. The first is its relation to the other flowers; i.e., remembering its "flower-array-dependent" (FAD) position. In human terms, this is somewhat akin to remembering, "It is the middle flower," or "It is the flower on the far right-hand side." The second possibility is remembering the position of the S+ relative to some aspect of the environment other than the unrewarding flowers. Previous literature has termed this "place learning" (Huber, Couvillon, & Bitterman, 1994) or memory for "absolute position" (Fiset & Doré, 1996; Fiset, Gagnon, & Beaulieu, 2000; Healy & Hurly, 1998), but to be more specific and indicate that the bee would remember the position of the S+ relative to something other than itself or the other flowers, we instead call this memory for the "flower-array-independent" (FAI) position. Finally, the bee could remember the position of the S+ relative to its current position in space. That is, it could use "position learning" (Huber et al., 1994) or "egocentric information" (Dyer, 1998; Gallistel, 1990; Fiset & Doré, 1996; Fiset et al., 2000; Shettleworth, 1998; Wang & Spelke, 2002). Using purely egocentric information to return to the S+ would involve remembering the direction and distance the bee must fly from its present position. However, the term "egocentric information" is vague because, as argued by Dyer (1998), all experience and representations of spatial relationships are fundamentally egocentric. Thus, to be more specific, in our situation we will replace the term egocentric information with "vector memory."

Which of the three types of information outlined above will the bees use to return to the S+? At present no theory exists to predict whether bees will use FAD, FAI, or vector information, but suggestions in favor of all three possibilities exist in the literature. For instance, honeybees returning to a goal have relied upon prominent objects close to the goal as landmarks (Cheng, Collett, Pickhard, & Wehner, 1987; Cheng, Collett, & Wehner, 1986). This suggests that bees in our experiments might use memory for the FAD position of the S+, as the unrewarding flowers are prominent objects close to the S+. On the other hand, if bees are like vertebrates, they will use FAI information to locate the position of the S+ (Brodbeck & Shettleworth, 1995; Fiset & Doré, 1996; Fiset et al., 2000). Even still, research on path integration (e.g., Collett & Collett, 2000) shows that bees are capable of calculating and flying along a vector between two points in space, suggesting bees will use a memorized vector. In addition, it is possible that bees can encode and use any combination of FAD, FAI, and vector information. Here, we have placed spatial cues in conflict to determine if bees make their choice based on one type of information over another.

The methodology used in our experiments was similar to that used by researchers studying vertebrate species (i.e., cats, Fiset & Doré, 1996; dogs, Fiset, et al., 2000; pigeons, Spetch & Edwards, 1988). Thus, in addition to gaining information on how bees encode a target, a comparative analysis can be facilitated. However, unlike the method used by Fiset and Doré (1996) and Fiset et al. (2000), the targets (flowers) in our experiments remained fixed in space throughout training. This difference can be justified for reasons of ecological validity: in nature flowers remain fixed in space, thus it is more ecologically valid to allow bees to obtain rewards presented in the same position across trials. In a similar vein, since cats and dogs prey upon other animals that move in space, it makes sense to present them with rewards that vary in position from trial to trial.

One idiosyncrasy of bumblebees worth noting is that ultimately experimenters cannot exert control over which bees will forage at any given time, and workers cannot be forced to forage (personal observation). Thus, the bees ultimately have control over whether they will be trained and tested, and when. In addition, training criteria reported in the bee literature varies from study to study, often with no rationale (e.g., Burmeister, Couvillon, & Bitterman, 1995). Thus, to address these problems the number of flower visits during training is included as a covariate in Experiments 1 and 2.

Our first experiment addresses whether bumblebees use the positions of the unrewarding flowers (S-) to locate the position of the S+. That is, do bumblebees make their flower choice based on memory for the FAD position of the S+? With similar methodology, cats (Fiset & Doré, 1996) and dogs (Fiset et al., 2000) tended to select the target-array-independent position of the S+, whereas pigeons tended to favor the target-array-dependent position (Spetch & Edwards, 1988). Additional issues specific to Experiment 1 are outlined below.

Experiment 1

The question of whether or not bumblebees remember the position of the S+ relative to the positions of the S- is also asking whether the S- are used as landmarks. There has been a great deal of research on landmark use by bees (Brown et al., 1997; Cheng et al., 1986; Cheng et al., 1987; Chittka & Geiger, 1995a; Chittka & Geiger, 1995b; Chittka et al., 1995; Dyer & Gould, 1983; Huber et al., 1994; Menzel et al., 1998), but it is difficult to predict exactly what object(s) bees, and other species, will select as landmarks (Etienne, Joris-Lambert, Dahn-Hurni, & Reverdin, 1995). Research by Cheng

(2000) and Chittka et al. (1995) suggest bees are partial to using prominent objects close to the goal, but "prominent" and "close" remain to be clearly defined. In our experiments, the S- are the most prominent objects closest to the S+, suggesting that they might be used as landmarks. If during testing bees are found to base their choice on memory for the FAD position of the S+, this indicates that landmarks do not have to differ physically from the goal, given that all targets in our experiments are identical in appearance.

In this experiment we have also considered the phenomenon of scent marking. Scent marks are chemicals, believed to originate from tarsal glands, which bees leave behind on flowers to supposedly act as an attractant or a repellant (Cameron, 1981; Chittka, Williams, Rasmussen, & Thomson 1999; Fry & Wehner, 2002; Giurfa & Núñez, 1992; Giurfa, Núñez, & Backhaus, 1994; Goulson, Chapman, & Hughes, 2001; Goulson, Hawson, & Stout, 1998; Kato, 1988; Schmitt & Bertsch, 1990; Schmitt, Lübke, & Francke, 1991; Stout, Goulson, & Allen, 1998; Thomson & Chittka, 2001; Wetherwax, 1986; Williams, 1998). There are many unanswered questions regarding the use and function of scent marks. For instance, it is unclear whether bees leave scent marks at every flower visit, or whether they can perceive a visual and/or tactile dimension of these marks (Thomson & Chittka, 2001). For bumblebees, scent marks have been hypothesized to function as a "probe-or-not-to-probe" cue: if a bumblebee revisits a flower that she has scent marked, she will probe it; if there is no scent mark, she will not probe (Cameron, 1981; Schmitt & Bertsch, 1990).

Currently the scent marking literature suffers in two respects. First, the interaction between scent marking and spatial memory has not been explored. Second, the complete elimination of scent marks in previous studies is questionable, as washing or ventilation was reported as methods to remove the chemicals (e.g., Cameron, 1981; Fry & Wehner, 2002; Schmitt & Bertsch, 1990). The only way to completely eliminate scent marks is to remove flower surfaces visited by bees and replace them with new, unused ones. Our study uses a novel methodology that addresses these concerns. One final point is that although scent marking has been treated as an independent variable, ultimately it is under the control of the bees. At present there is no way for humans to directly observe whether or not a bee has placed a scent mark on a flower, and there is also no current method for the experimenter to deposit scent marks onto flowers. The most an experimenter can do is set the conditions for scent marking to occur, and observe the bees' behaviour when presented with a presumably scent marked flower. For these reasons, distinguishing conditions on the basis of "scent mark" or "no scent mark" can be misleading; instead, conditions in which scent marks are allowed are termed conditions with "old flower covers," and conditions where possible scent marks are eliminated are termed conditions with "new flower covers."

In this experiment, bumblebees are exposed to two artificial flowers, one of which offers sugar solution (S+), the other offering nothing (S-). After learning to probe the S+ for reward and avoid the S-, bees are tested with empty flowers. Testing is done using a 2 (flowers moved or unmoved at testing) x 3 (nature of flowers: old covers unmoved, old covers moved, or new covers) between-subjects design (see Figure 2). In the conditions where flowers are not moved (left column of Figure 2), bees are expected to select the flower that is in the same position as the S+ in training (the flower below the arrow in the left column of Figure 2). They can use memory for FAD and/or FAI position of the S+ to do so. Conditions where flowers are moved (right column of Figure 2) pits memory for

FAD position of the S+ against memory for FAI position of the S+. Here, the test flower in the S- position is moved to the other side of the test flower in the S+ position. If bees use memory for FAD position of the S+, they should choose the moved flower; i.e., the flower that is now in the same FAD but wrong FAI position as the S+ in training. If bees use memory for FAI position, they should choose the flower beneath the arrow in Figure 2. Whichever test flower the bees choose, they should probe it in the conditions where the cover is allowed a scent mark (i.e., old flower covers are used).

Method

Subjects

One hundred forty-four worker bees from a colony of bumblebees (*Bombus impatiens*) were used (24 bees per condition). The colony was obtained from Biobest Canada Ltd. (Leamington, Ontario). Worker bees were labeled using colored, numbered, plastic discs glued to the bees' thorax. Pollen was provided in the colony *ad libitum* throughout the study. They were supplemented with sugar solution but the amount was restricted during training and testing to stimulate foraging.

Flight Cage and Housing

The colony was housed in a plastic container within a styrofoam-insulated cardboard box (30 cm x 15 cm x 15 cm), connected to a wooden corridor (30 cm long), which was in turn connected to a wooden framed, screened flight cage (183 cm x 188 cm x 190.5 cm). The corridor was covered with removable glass plates to permit observation of bees entering and exiting the colony and flight cage. Two vertical plastic gates were inserted between the glass plates of the corridor so that the experimenter could control which bees entered and exited the colony and flight cage. To aid bees in returning to the colony, inside the flight cage a small disc with radiating black stripes was placed around the hole to the corridor. The floor of the flight cage was painted green and contained evenly spaced holes in which artificial flowers could be inserted. Illumination was provided by fluorescent lights on the ceiling of the room as well as above the ceiling of the flight cage. The average temperature of the room was 20°C (range: 18°C-22°C).

Artificial Flowers

The artificial flowers were based on those used by Hartling and Plowright (1979; see Figure 1). Each flower consisted of an open-top wooden box (12.5 cm long x 8 cm large) supported on a rod (60 cm high). The wooden box was covered by a yellow, thin piece of plastic (duraflex) that could be easily removed and replaced. This plastic piece is referred to as the flower cover. Each flower was connected to a 12-V battery-powered switchboard located outside of the flight cage.

Nectar level of the rewarding flower was not visible to the bees. The inner nectar well held approximately 5 ml of sugar solution (2 parts table sugar to 1 part water by volume). Pressing the flower's corresponding button on the switchboard filled the capillary tube with sugar solution ($M = 2.69$ μL, $SE = 0.19$ μL; Hartling & Plowright, 1979), which could be depleted in one bee visit. Bees accessed the sugar solution by probing the "nectar hole" in the flower cover, and thus probing the capillary tube (see Figure 1). The time needed to press the button and refill the capillary tube was approximately one second (Hartling & Plowright, 1979). A small white ring with radiating blue stripes was placed around the nectar hole to act as nectar guides. The Teflon plate with attached capillary tube can be removed; separate Teflon plates were used for training and testing.

Two artificial flowers were placed in the flight cage side-by-side, 20 cm apart, such that when the bees entered the flight cage they encountered a row of flowers 4 cm away from, and parallel to, the left-hand wall of the flight cage. One set of flowers was used only for training and another set was used for testing.

Procedure

Training and testing procedures for all conditions are illustrated in Figure 2. The position of the S+ was randomly assigned at the beginning of the experiment, and once all data were collected for that S+ position the other flower was then assigned as the S+ and data were collected for that position. Each subject was randomly assigned to one of the six test conditions.

Each day of the experiment proceeded as follows: (1) a free-foraging session, in which new foragers were identified; (2) bees were trained to find the nectar hole of the flower; (3) the pre-test, in which bees were allowed to visit the rewarding flower once; (4) testing, in which bees were exposed to one of the experimental conditions.

Free-foraging and training. To facilitate foraging, colonies were not fed sugar solution one to two days before training so that all surface honey pots in the colony were empty. During free-foraging, one vial cap of honey solution (2 parts commercial honey to 1 part water by volume) and several vial caps of sugar solution (2 parts table sugar to 1 part water by volume) were placed on top of the S+ to allow bees to find the S+ using olfactory cues (each vial cap 2.5 cm diameter, approximately 5 ml). Honey solution can be detected by scent, whereas sugar solution cannot (Brown & Demas, 1994; Couvillon & Bitterman, 1991; Giurfa et al., 1994). The gates of the corridor were removed and the experimenter recorded the identity of the foragers, i.e., bees that repeatedly collected honey solution from the S+ and returned to the colony. Once one or more foragers were identified this training step ended and bees remaining in the flight cage were returned to the colony.

Next, the identified foragers were trained to probe the nectar hole of the S+ without the aid of scent. The button on the switchboard corresponding to the S+ was pressed, filling its inner capillary tube with sugar solution. One small drop of honey solution was placed on the flower cover and several large drops of sugar solution were placed closer to the nectar hole. Previously identified bees were released into the flight cage as they entered the corridor. The experimenter recorded all flower visits for each bee. Once the drop of honey solution was depleted it was not replenished, but the sugar solution drops were always replenished. When bees began to consume the drops of sugar solution after depletion of the honey solution drop, the flower cover of the S+ was replaced with a new cover so now there would be no trace of honey scent on the S+. Drops of sugar solution were placed around the nectar hole. After the drops were depleted bees often swiped their proboscis across the surface of the flower; the experimenter observed whether during this process the bee found the nectar hole. When a bee found and subsequently probed the nectar hole it was readily observable: her proboscis visibly went down into the hole and the bee remained stationary for several seconds while she imbibed the sugar solution. The capillary tube of the S+ was filled for each bee visit, thus giving each bee a reward on each visit to the S+. The number of times each forager probed the hole was recorded. This training period stopped after one or more foragers were observed probing the S+ and their visits to the S- decreased.

Pre-test and testing. All flower covers were replaced with new, unused covers. The experimenter pressed the switchboard button corresponding to the S+, filling the flower's capillary tube with sugar solution, and the inner vial cap of sugar solution was removed. Once a bee that was observed consistently probing the S+ during training entered the corridor, she was released into the flight cage. She was allowed to probe the S+ and extract all of the sugar solution in the capillary tube. Once she had drained the capillary tube and left the S+, the pre-test ended. At this point the only possible odour or residue on the flower cover would be the individual forager's ostensible scent mark left after probing.

Immediately after the forager probed and alighted from the S+, the experimenter turned off all lights in the testing room except for one red light. This was done to limit the bee's flight in the cage as the experimenter entered the cage to manipulate the flowers. Flowers were manipulated according to one of six test conditions (see Figure 2). The experimenter also replaced the Teflon plate and capillary tube with a set that had never exposed to honey or sugar solution. Once the experimenter had manipulated the flowers and exited the flight cage, the lights were turned back on and the red light was turned off. (The time required to turn off lights, manipulate the flowers, and turn lights back on was between one and two minutes.) Once the lights were back on, the bee in the cage resumed flight and the experimenter recorded her first flower choice as an approach, land, or probe. An approach was defined as flying 2 cm or less above the nectar hole and turning away without landing. The landing response was contact of the bee's feet on the flower and the bee did not probe the hole. Finally, the probe response required landing on a flower and probing its nectar hole (Schmitt & Bertsch, 1990). All observations are independent since each bee was only allowed one choice during testing.

Results

Two analyses were conducted. The first was a χ^2 test for goodness of fit to compare frequency of choice by bumblebees for FAI position to chance (i.e., .50). Choice frequencies are displayed in Table 1. The second was a logistic analysis for binomial proportions using GLIM version 4.0 statistical software (Francis, Green, & Payne, 1993). This investigated the influence of variables such as proportion of rewarding visits during training and assignment of S+ during training on choice by bumblebees for FAI position of the S+. The factors of interest, nature of flowers (i.e., old covers unmoved, old covers moved, or new covers) and flowers moved or not, were entered last into the linear model. For these analyses choice by bumblebees for FAI position was assigned a value of 1, whereas choice of other position was assigned a value of 0. Frequency of probing behaviour was investigated in a similar manner.

Comparisons with a Theoretical Value of Chance

In each of the six test conditions, bees chose the FAI position at frequencies significantly higher than chance. See Table 1 for χ^2 values.

Effects of Experimental Variables

Flower choice. Assignment of S+ in training ($\chi^2(1, N = 144) = 0.16, p > .05$) and nature of flowers during testing ($\chi^2(2, N = 144) = 1.58, p > .05$) did not have significant influences on test choices. However, there was a main effect of mean proportion of rewarding visits during training ($\chi^2(1, N = 144) = 5.32, p < .05$). In general, the higher the mean proportion of rewarding visits during training, the higher the choice frequency for FAI position. There was also a main effect of whether or not flowers were moved during testing. Choice frequency for FAI position was higher if flowers were not moved ($\chi^2(1, N = 144) = 8.88, p < .05$), showing that bees were more likely to choose the flower in the FAI position if it was also in the FAD position.

Response type. Probe responses were assigned a value of 1 and landings and approaches a value of 0. The factors of interest, nature of flowers (i.e., old covers unmoved, old covers moved, or new covers), flowers moved or not, and flower choice (FAD or FAI) were entered last into the linear model. Proportion of rewarding visits during training ($\chi^2(1, N = 144) = 0.26, p > .05$), assignment of S+ in training ($\chi^2(1, N = 144) = 2.74, p > .05$), flowers moved or not ($\chi^2(1, N = 144) = 0.74, p > .05$), and test flower choice ($\chi^2(1, N = 144) = 0.13, p > .05$) did not have significant influences on probing frequency. However, there was a main effect of nature of the flowers ($\chi^2(2, N = 144) = 8.92, p < .05$): When bees landed on a flower with an old cover they were most likely to probe (Conditions 1 and 2).

Discussion

When given the choice between a test flower in the FAD position and a test flower in the FAI position of the S+, bumblebees chose the latter. Interestingly, similar results have been obtained from cats (Fiset & Doré, 1996) and dogs (Fiset et al., 2000) in comparable laboratory situations. Nevertheless, the decrease in choice frequency for FAI position when test flowers were moved suggests that bumblebees encoded the FAD position of the S+. In sum, when the position of the S- is placed in conflict with other external cues, these other cues take precedence.

Consistent with previous research (Cameron, 1981; Schmitt & Bertsch, 1990), probing frequency decreased when old covers were replaced with new ones during testing (i.e., when all possible scent marks were eliminated). Presumed presence of scent marks affected behaviour once on the flower and not flower choice.

In Experiment 2 we will omit the influence of scent marks by using new, unused flower covers in all test conditions, thereby restricting the analysis to the use of spatial information, the main focus of this study. One additional methodological change for Experiment 2 is the exclusion of the approach response as a dependent variable. In Experiment 1 the probing and landing responses provided more decisive results: in each test condition the number of bees that landed or probed was always higher than the number of bees that only approached; and, for those bees that approached, their choices were always in the same direction as the data for lands and probes. Therefore, only the land and probe responses will be included in Experiment 2. It is predicted, however, that because scent marks will be eliminated during testing, probing behavior will most likely decrease and landing will become the most frequent response.

In summary, in Experiment 1 bumblebees remembered the position of the S+ using memory for some cue other than the S+ position relative to the S-. As noted by Fiset and Doré (1996) and Fiset et al. (2000), the animal can achieve this by using allocentric information (referred to here as FAI information) or egocentric information (referred to here as vector memory). Thus, to determine the type of information preferred by bumblebees, Experiment 2 will place FAI information and vector memory in conflict.

Experiment 2

In Experiment 1, bees could have located the FAI position of the S+ using memory for a flight vector of particular distance and direction from the colony. Alternatively, the FAI position of the S+ could have been located using memory for its position relative to some other external cue. The bees might use a variety of such external cues; in Experiment 1 we showed that they used cues other than the neighboring flowers. A major disadvantage of using strictly vector memory is that if the animal were displaced for some reason (e.g., fleeing a sudden predator, being blown off course by winds), the stored direction and distance information would now lead the individual to the wrong location. FAI information permits the animal to reorient itself, provided the chosen reference object(s) remain fixed in space.

In Experiment 2 bumblebees were again exposed to an array of artificial flowers, one of which offered reward (S+). However, once a bee had learned to probe this flower for nectar, testing involved shifting the position of the colony and arranging the artificial flowers in the cage such that when the bee re-emerged to forage she now had a choice between two flowers: one flower would be chosen if the bee used vector memory to remember the position of the S+, and the other flower would be chosen if FAI information was used (see Figure 3).

Method

All aspects of the methods were the same as in Experiment 1 except for the following.

Subjects

Fifty worker bees from two new colonies of bumblebees (*Bombus impatiens*) were obtained from Biobest Canada Ltd. (Leamington, Ontario).

Artificial Flowers

Three flowers were used during training and two during testing. Flowers were placed 34 cm apart, in a row 12 cm away from, and parallel to, the back wall of the cage opposite the colony. The outer flowers were 42 cm from the sides of the cage (see Figure 2). Bees had to fly approximately 170 cm from the colony to the flower array. Flowers were in a different position in the cage compared to Experiment 1 because only one cage wall could accommodate the three possible colony positions, and the flight cage could not be moved within the laboratory.

Procedure

Training. A 3 (S+ positions) x 3 (colony positions) between-subjects design was used. One of the three artificial flower positions was randomly assigned as the S+, and the colony was placed in one of three randomly assigned positions, across from one of the three flowers (see Figure 3). Note that in Figure 3 the S+ is in front of the colony, but the S+ could also be at a diagonal from the colony. After test data were obtained for that colony and S+ combination, the positions of the S+ and colony were randomly reassigned. This process was repeated until data were obtained for all S+ and colony positions. Once a bee was observed probing the S+ and her visits to the S- decreased, she was ready to be tested.

Testing. When a bee to be tested emerged from the colony she was kept between the two gates of the corridor. The experimenter shifted the corridor and colony to one of the other (randomly chosen) colony positions. For bees in the control condition the colony and corridor were shifted the same distance as the other test positions, but was ultimately returned to the same position as in training. The corridor entrance guide (disc with black radiating stripes) in the flight cage was moved to the entrance corresponding to the new colony position. All artificial flowers in the cage were removed and replaced with two flowers identical to those used in training. These flowers had new, unused covers so that scent marks were absent. One flower was placed in the position that would be chosen if the bee based her choice on FAI information, and the other flower was placed in a position corresponding to the choice that would be made if vector memory was used (see Figure 3). For the control condition, one flower was placed in the same position as the S+ and the other flower was randomly assigned to one of the positions occupied by an S- during training.

Once the flowers were manipulated and the colony and corridor moved, the bee was released into the cage and her first three flower choices (land or probe) were recorded. After her third choice testing ended. Testing was terminated if she did not make three choices after 10 minutes.

Results

Landing was the most frequent response type in both the Control Group and the Test Group. For the first test choice, only one bee in each group probed their chosen flower.[2]

Comparisons with a Theoretical Value of Chance

Control group. One bee failed to make any flower choice during testing. The majority of bees in this group chose the flower that was in the same position as the S+ in training (see Table 2), though the effect was not significant: $\chi^2(1, N = 17) = 2.88, p > .05$. The FAI position was chosen above chance levels for the second choice ($\chi^2(1, N = 15) = 8.07, p < .05$) and exclusively for the third choice. Four of the five bees that did not choose the FAI position for their first choice switched to choosing this position for their second and/or third choice.

Test group. One bee did not make any flower choice. For the remaining bees the FAI position was chosen above chance levels for the first choice ($\chi^2(1, N = 31) = 14.23, p < .05$; see Table 2), second choice ($\chi^2(1, N = 28) = 7.00, p < .05$), and third choice ($\chi^2(1, N = 23) = 5.26, p < .05$).

It is possible that bees trained with the S+ in one of the outer positions could have used memory for FAD position of the S+ when they made their test choice (see Figure 3).

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[2] For the second and third test choices, frequencies of test flower choices and number of probe responses are found in Appendix B.

Thus, results from bees trained with the S+ in the middle of the array were analyzed separately because during testing there was no longer a middle flower. Consistent with the results above, FAI position was chosen significantly more often than chance for the first ($\chi^2(1, N = 11) = 4.46, p < .05$), second ($\chi^2(1, N = 10) = 6.40, p < .05$), and third (all 9 bees) choices. This suggests memory for FAD position of the S+ was not used in flower selection during testing.

Effects of Experimental Variables

The factors colony (i.e., colony 1 or 2; $\chi^2(1, N = 48) = 0.45, p > .05$), proportion of rewarding visits in training ($\chi^2(1, N = 48) = 1.55, p > .05$), position of colony in training ($\chi^2(2, N = 48) = 1.80, p > .05$), position of S+ in training ($\chi^2(2, N = 48) = 0.04, p > .05$), and position of colony during testing ($\chi^2(2, N = 48) = 0.82, p > .05$) had no significant influence over choice of FAI position. A significant interaction was found between position of S+ in training and position of colony during testing: $\chi^2(4, N = 48) = 9.52, p < .05$. The highest choice frequency for FAI position ($M = 1.00$, $SD = 0.00$) occurred in two instances: (1) when the S+ was in position 2 in training and the colony was in position 2 in testing; (2) when the S+ was in position 3 in training and the colony was in position 3 in testing. The lowest choice frequency for FAI position ($M = 0.50$, $SD = 0.50$) occurred when the S+ was in position 2 in training and the colony was in position 3 in testing. Finally, there was no significant interaction between the positions of the S+ and colony in training ($\chi^2(3, N = 48) = 4.17, p > .05$), the position of the colony during training and testing ($\chi^2(4, N = 48) = 9.01, p > .05$), and no significant three-way interaction between positions of the S+ and colony in training and position of the colony in testing ($\chi^2(6, N = 48) = 3.67, p > .05$).

A comparison of the choice frequency for FAI position of the Test and Control groups showed no significant effect of condition on choice of FAI position ($\chi^2(1, N = 48) = 0.00, p > .05$). Thus, bees in the Test Group did not choose FAI position more often than bees in the Control Group, and vice versa. (It should be noted that bees in the

Control Group could use FAI information or vector memory to select the flower in the FAI position.) Choice frequencies for FAI position were 0.71 for the Control Group (*SD* = 0.47) and 0.84 for the Test Group (*SD* = 0.37).

Discussion

In this experiment there was a significant tendency for bees to choose the test flower that corresponded to the use of FAI information. This ruled out the possibility that bees used simply a memorized flight vector. The specific cues the bees chose as FAI information remain to be discovered. One interesting possibility, as described by Dyer (1998), is that the bees used a combination of FAI information and egocentric representations to locate the position of the S+. The bees might have stored a retinal image of whatever external object(s) they used to locate the position of the S+. When returning to the goal they could have used some external directional reference to achieve the proper orientation for image matching between "retinal snapshot" and percept. In this case, since the directional reference is simply used to orient the bee so it can use its egocentric memories efficiently, the distinction between egocentric and FAI coordinate systems becomes blurred. Thus, what is known as the egocentric-allocentric dichotomy should be reexamined (Dyer, 1998).

Next, the reasons for the interaction between rewarding flower position in training and colony position during testing are unknown. For instance, it is not known why, when the S+ was in position 2 or 3 in training, placing the colony directly in front of the FAI flower in testing would be an advantage for selecting it. We also do not know why placing the S+ in position 2 in training and placing the colony in position 3 in testing would hinder bees' choices. This is particularly curious given that position of the colony during training appears to have exerted no significant influence. Whatever FAI cue(s) the bees used to encode the S+, the position of the colony during testing (and thus the location at which the bee entered the cage) might have made these cues more or less salient during flower selection.

Finally, no scent marks would have been available during testing in this experiment. In agreement with Cameron (1981) and Schmitt and Bertsch (1990), most bees were observed landing on, but not probing, their test flower.

General Discussion

How did bumblebees in our experiments encode the position of the rewarding flower? First, evidence for memory for FAI position of the S+ was found in both experiments. Second, moving the S- resulted in a lower choice frequency for FAI position, indicating that the FAD position of the S+ was encoded and exerted some influence on flower selection. Yet because the FAI position was still chosen above chance levels, the S- was not used in isolation to locate the S+ despite the fact it was a prominent object close to the goal and the only other object in the cage. The bees used some other cue in conjunction with the S- to make their choice. Third, probing behaviour had no influence on flower choice and occurred at lower frequencies when scent marks were eliminated, corroborating past research. Finally, when given a choice, FAI position was used over a memorized flight vector in flower selection.

What cues besides the positions of the unrewarding flowers did the bees use to remember the position of the S+? One possibility is the bees remembered the relationship between the position of the S+ and the global configuration of the flight cage (Fiset & Dore, 1996). Past research (Cartwright & Collett, 1982, 1983; Cheng, 1986, 2000; Collett & Cartwright, 1983; Dyer, 1998; Gallistel, 1990) suggests that during training the bees would take a "panoramic snapshot" of the general surroundings once they are near the

S+. This snapshot, rather than being a complete carbon copy of what was on the retina, is likely a processed version emphasizing edges (Collett, 1996). During testing, to relocate the S+ bees would attempt to match this stored template with what is perceived on the retina, and restrict their search area to where discrepancy between template and percept is minimal. If our bees used this template-matching strategy, they would have encountered miss-matches between template and percept in Experiment 1, in conditions where flowers were moved (see Figure 2), and in Experiment 2, which involved completely removing one flower during testing (see Figure 3). Specifically, aligning the template and percept of the cage might result in misaligning the template and percept of the flower array, and aligning the template and percept of the flower array would result in misaligning the percept and template of the cage. Since our results show that bees consistently chose the FAI position of the S+, thereby disregarding the position of the S+ relative to the positions of the other flowers, this suggests that if template matching was used, aligning the percept and template of the cage took precedence over aligning the percept and template of the flower array. In other words, matching the larger aspects of the template was preferred over matching its smaller features.

Another possibility is that the bees located the S+ using the distance between the S+ and the walls of the cage (Fiset & Doré, 1996; M. Shapiro, personal communication, March 25, 2004). For instance, Cheng and Sherry (1992) showed that chickadees and pigeons located a target using the perpendicular distance from an edge to the goal. If bees encoded the distance from the walls to the S+ they must have done so quite accurately, considering the S- and S+ were separated by only 20 cm. Our results do not allow us to conclude whether the bees encoded absolute position of the S+ by template-matching, by estimating distance, both, or none of the above.

One important finding from our experiments is that the S-, despite being identical in appearance to the S+ and the closest and most prominent object to the S+, was not relied on as a landmark. This leads us to conclude that the properties or placement of an object cannot at present predict whether that object will be used to locate a goal. "Landmarkness" can only be inferred by the animal's behavior. Further research should delineate under which conditions objects are and are not used as reference points to locate sources of food. The present study represents a step in this direction.

In conclusion, our experiments provide a glimpse into the contents of bumblebee spatial memory and allow for interesting cross-species comparisons with research that has used similar methodology (e.g., Fiset & Doré, 1996; Fiset et al., 2000; Spetch & Edwards, 1988). Future research might investigate whether other forms of spatial information that were not used by bumblebees here can nevertheless be encoded and used under different circumstances. For instance, do bumblebees encode and use FAD position if FAI information is made unavailable or unreliable? Can they rely upon vector memories if they cannot use FAI information? Perhaps, as suggested by research that has addressed these specific questions with a number of species (e.g., Collett & Collett, 2000; Fiset & Doré, 1996; Fiset et al., 2000; Goto, Wills, & Lea, 2004; Spetch & Edwards, 1988), strategies can be adjusted according to situational demands.

Acknowledgements

This paper was prepared as part of the first author's doctoral thesis. Experiment 1 was presented at the 13th Annual Meeting of the Canadian Society for Brain, Behavior, and Cognitive Science, Hamilton, Ontario, June 2003, and Experiment 2 was presented at the 11th Annual Conference on Comparative Cognition, Melbourne, FL, March 2004. This research was supported by a research grant to C. M. S. P. and a graduate scholarship to D. L. C. from the Natural Science and Engineering Research Council of Canada. We thank Alain Desrochers, Sylvain Fiset, Sylvain Gagnon, and Pierre Mercier for constructive advice on the design of the experiments and the manuscript. Both experiments comply with the standards of the Canadian Council on Animal Care.

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Table 1.

Observed Frequencies for Experiment 1 for Choices for the Flower in the Flower Array Independent (FAI) Position (versus the Other Flower) Out of N = 24 Choices

	FAI Position	χ^2	*p*	Probing Frequency
		Flowers Unmoved		
Condition 1:				
Old Covers Unmoved	21	13.50	.0003	21
Condition 3:				
Old Covers Moved	23	20.17	.00001	11
Condition 5:				
New Covers	24	24.00	.000001	13
		Flowers Moved		
Condition 2:				
Old Covers Unmoved	17	4.17	.04	19
Condition 4:				
Old Covers Moved	18	6.00	.01	15
Condition 6:				
New Covers	19	8.17	.004	14

Note. For each condition *df* = 1 and expected frequencies according to chance are 12:12.

Table 2.

Observed Frequencies of Test Flower Choices, Expected Values According to Chance, and Number of Probe Responses for Experiment 2

	S+ Position	Other Position	*p*
		Control Group	
Observed	12	5	.09
Expected	8.5	8.5	
	FAI Position	**Vector Memory Position**	***p***
		Test Group	
Observed	26	5	.0002
Expected	15.5	15.5	
		Test Group, Middle Flower S+	
Observed	9	2	.035
Expected	5.5	5.5	

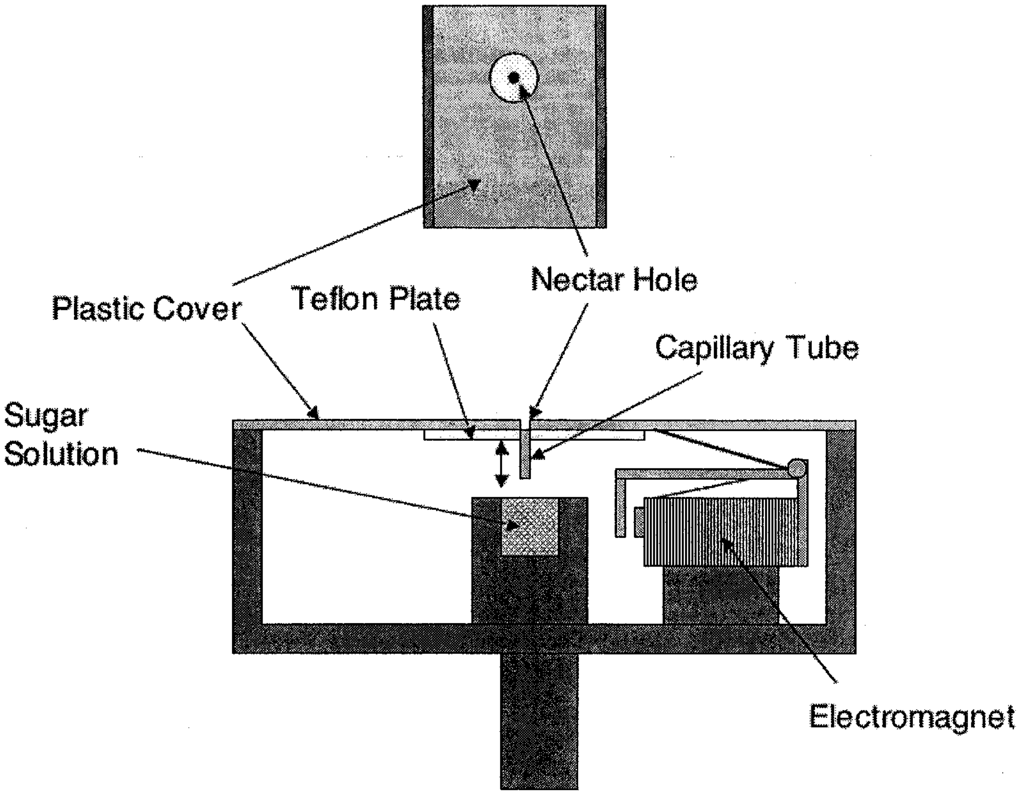
Note. For each condition *df* = 1. For the Control Group, S+ position can be chosen using FAI information or vector memory. FAI = Flower Array Independent.

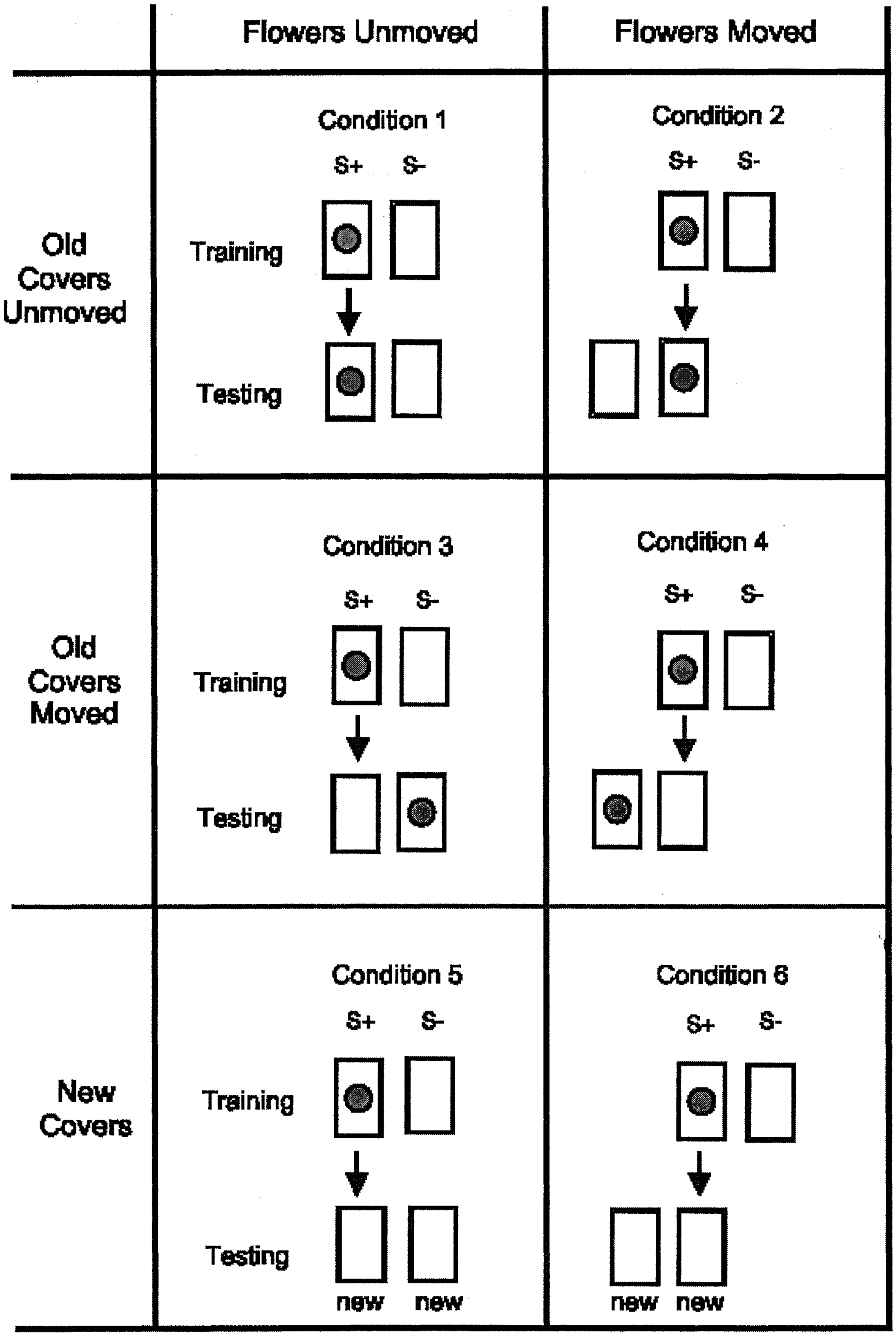
Figure Captions

Figure 1. Bird's eye view (top) and cross section (bottom) of an artificial flower used in all experiments. Bidirectional arrow indicates the action when the flower's corresponding button on the switchboard is pressed and released. Pressing the button lowers the Teflon plate, dipping the capillary tube into the sugar solution. Releasing the button returns the Teflon plate, with capillary tube filled with sugar solution, to its original position up against the underside of the flower cover. Redrawn with permission.

Figure 2. Example of training and testing procedures in Experiment 1. S+ = rewarding flower (containing sugar solution); S- = unrewarding flower (containing nothing). Each bee was trained with the S+ in one of the two positions. The positions of the S+ and S- were counterbalanced across subjects. Circle on S+ = presumed scent-mark deposited after bee extracts sugar solution. In the left column, the flower below the arrow is in the same FAI and FAD position as the S+. In the right column, it is in the FAI position but not the FAD position.

Figure 3. Example of training and testing procedures in Experiment 2. S+ = rewarding flower (containing sugar solution); other flowers (small squares) contain nothing. Individual bees were trained with the S+ and colony each in one of three positions (1, 2, or 3). Positions of the S+ and colony were counterbalanced across subjects. In the training examples, dotted outlines show the other two possible colony positions. Testing involved moving the colony to one of these two other positions, except in the Control condition where the colony remained in the same position but was shifted over and back again an equal distance (bidirectional arrow). In the Control and Test examples, dotted lines indicate positions previously occupied by flowers and/or colony in training. FAI = flower choice if flower-array-independent information used; V = flower choice if vector memory used exclusively. Drawing not to scale.


Plastic Cover
Teflon Plate
Nectar Hole
Capillary Tube
Sugar
Solution
Electromagnet


Flowers Unmoved
Flowers Moved
Old Covers Unmoved
Condition 1
S+
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Training
Testing
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S-
Old Covers Moved
Condition 3
S+
S-
Training
Testing
Condition 4
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Condition 6
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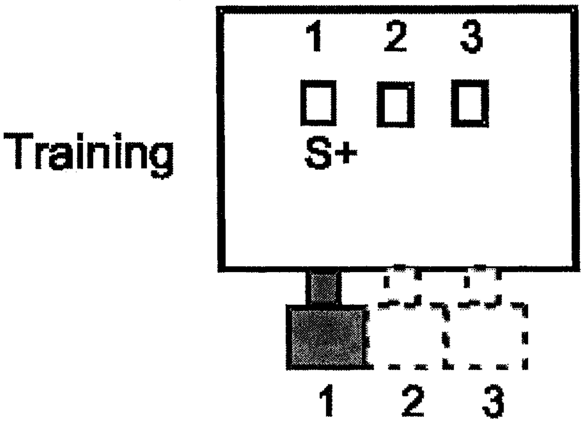
Training
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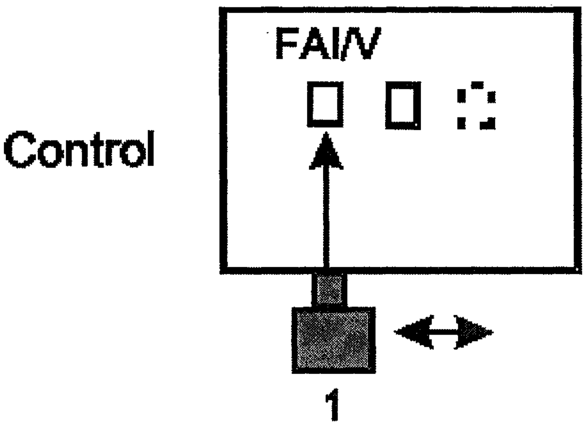
S+
1 2 3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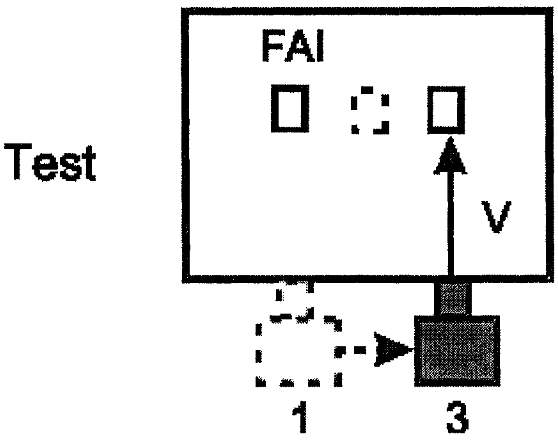
1


Test
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3

CHAPTER FOUR

General Discussion

Contributions and What Was Learned

The drawing of the three. Three specific questions were posed at the beginning of this thesis. First, can unrewarding flowers function as landmarks? When an unrewarding flower was the only other object in a flight cage, changing its position relative to the S+ had an effect on bees' choices. Specifically, preference for the FAI position dropped, indicating that the position of the S- in training was encoded. However, since the FAI position was still chosen above chance levels, some other cue was of more importance than the position of the S- during flower selection.

The question of whether the unrewarding flowers functioned as landmarks essentially asks what the definition of a landmark is from a bee's perspective. What object(s) or feature(s) of the environment will a bee rely upon to aid her back to a source of nectar? Although the present results in combination with the past research on landmarks (e.g., Cheng, 2000; Cheng et al., 1986, 1987; Chittka et al., 1995) do not presently allow for precise predictions, they at least allow us to assume that the bee will not likely rely on an object for landmark purposes that is similar to the goal in colour and shape, even if it is the closest and prominent object near the goal. Whether the artificial flowers used in this thesis are considered to represent separate flower patches or individual flowers within a patch in a natural environment, one reason they would not be used as landmarks is that natural flowers change appearance and die over time, rendering them unreliable for locating a specific point in space. Choosing nearby objects with colours and shapes different from flowers may increase the chance that it is a more stable entity, such as a rock or a lawn ornament. An even more stable cue may be the general configuration of the surroundings, such as the geometry of the flight cage or of a garden or farmer's field.

The second question posed at the beginning of this thesis is: How do bumblebees encode an array of artificial flowers in a laboratory setting? Specifically, do they encode the absolute (FAI) or relative (FAD) position of the rewarding flower? As discussed above, the answer is a bit of both. Although bees showed a clear preference for the flower in the correct FAI position, selection of the FAI flower dropped when the test flower in the correct FAI position was also in the incorrect FAD position. FAI position was selected using some cue outside of the flower array and not by using a memorized flight vector from colony to S+.

Finally, what do the present findings contribute towards understanding how scent marks are used, particularly how they interact with spatial memory? For the most part, presumed presence of scent marks functioned as a "probe-or-not-to-probe" cue, confirming Cameron's (1978, 1981) hypothesis. However, when scent marks were eliminated in Experiment 2 of Chapter 2, flower choice dropped to chance levels indicating that presumed presence of scent marks affected choice behaviour and not simply behaviour once on the chosen flower. The clearest conclusion to be made from these results is that a means by which to directly observe and manipulate scent marks must be found if their function is ever to be elucidated. This applies to both laboratory and field studies. Once such means are discovered, this thesis provides a method by which to address the question of whether scent marks are a form of bee memory.

Strengths of this Thesis

Worthy of note is that a complex apparatus was not required to learn what is discussed above. Each experiment was conducted using a colony of bumblebees, a flight cage, and several artificial flowers. This relatively simple apparatus allowed for an analysis of bumblebee behaviour and memory that spans the length of this thesis. As well, using an experimental approach allowed for behaviour and answers and that might not be seen or obtained using less controlled environments.

Another strength of this thesis is that it included questions about bumblebee behaviour that have not been addressed together before. Specifically, spatial memory and scent marking have been addressed as separate phenomena in the literature. Here the idea was entertained that scent marking could be part of the bee memory system, and a novel methodology allowed scent marking and spatial memory to be studied simultaneously. In addition, whether bumblebees encode and use relative position, absolute position, allocentric information, and egocentric information is a question that had been neglected in bee research. By using a paradigm similar to those used with vertebrates to explore the same concepts (see Fiset & Doré, 1996; Fiset et al., 2000; Spetch & Edwards, 1988), a comparative analysis was facilitated. Finally, the definition of the oft-used term "landmark" was called into question, and the issue of the difficulty in predicting what objects an animal will use to return to a place was brought to the fore.

Limitations of this Thesis

Undoubtedly the experiments here are limited in the degree to which they explain what happens in nature: Bumblebees were restricted to an indoor flight cage deprived of the usual variety of stimuli encountered outdoors by a free-flying bee. Given that the flight cage presented the bees with a foraging environment of very limited size and stimulus variety compared to that possible in the wild, the degree to which the results generalize to natural conditions could be questioned. That is, given a larger and more varied foraging range, bumblebees may not necessarily show a bias for remembering a goal's absolute (FAI) position using allocentric cues. In fact, previous research (e.g., Cheng, 2000; Collett, 1996; Collett & Collett, 2000) has placed an emphasis on long-distance goal finding, where bees are required to fly quite far from the colony, ergo more closely mimicking natural situations, and has found reliance on both egocentric and allocentric information. However, much of this research involved honeybees, which are known to travel long distances from the nest to obtain food: 1-6 km, sometimes as far as 20 km (von Frisch, 1967). Although it has been assumed that bumblebees would forage as close to their colony as possible to minimize energy costs, the foraging ranges of bumblebees have yet to be clearly quantified (Goulson, 2003). Thus, it is not certain to what degree the size of the bumblebees' foraging range in the flight cage deviates from natural conditions. Interestingly, in the last experiment, bees were rarely seen flying straight from the colony to the test flowers; they often flew around the cage and/or up to one or more of the corners of the cage before making a choice. Perhaps the steps used in long-distance foraging (i.e., first striking a vector, then beaconing in on a landmark, then image matching; see Cheng, 2000) may have been used here by the bumblebees, but on a smaller scale.

Future Directions

"Probing" for spatial memory in bumblebees. Researchers working with nonhuman species have the particular problem of deciding upon a dependent variable that is both within the species repertoire and is the most accurate expression of the concept under study. In other words, it might make our lives much easier if we could just verbally ask the bee how she spatially encoded the S+, and she could speak her answer. In the absence of this luxury, this thesis used the bees' first test flower choice as an expression of spatial memory, choice being defined as probing, landing on, or approaching the flower. The question must be asked: can a bumblebee's spatial memory for the S+ be completely and accurately expressed through her first flower choice?

The reasoning behind using a bee's first flower choice was that bumblebees were trained until they probed the S+ consistently; if they remembered the location of the reward they should choose that location immediately after entering the cage. One measure that may provide additional insight is flight density: Do bees concentrate their flying more in some areas of the cage compared to others? Fry and Wehner (2002) have measured flight density in their experiments on spatial memory in bees. The process involves videotaping the bee's flight then applying special digitizing software to obtain a density distribution. The advantage of measuring flight density is it allows for possible choices beyond the options (i.e., the flowers) provided by the researchers. For instance, in a follow-up to the last experiment presented in this thesis, testing could involve presenting a flower in the vector memory position and another flower in neither the vector memory position nor the FAI position. The question here would be: Would bees select the vector memory flower if no flower were in the FAI position? In this case, measuring flight density along with the bee's first choice would be worthwhile, considering the bees may still rely on FAI information but there is no flower to choose in that location to demonstrate this. Bees may resort to hovering in the area corresponding to the use of FAI cues. In sum, measuring—and discovering—multiple forms of response will help in acquiring a more complete picture of what an animal remembers about the space around it.

A "multi-modal" approach to learning more about bee memory contents. One exciting conclusion that has been made from the decades of research with bees is that these creatures, with such minute nervous systems, are capable of learning and forming memories. Of course, such a small brain, in combination with the performance boundaries set by physiology and morphology, carries various cognitive and behavioural limitations (Bitterman, 1996; Menzel, 1990). For instance, "bees are helplessly 'stupid' when trying to escape from a room, and will readily crash into a window [repeatedly], but are surprisingly 'inventive' in handling a flower to extract nectar or collect pollen" (Menzel, 1990, p. 284). Thus, a bee's hypothesized cognitive "toolkit" (i.e., the ability to form memories for flight vectors, landmarks, and retinal images; Collett, 1998, Collett & Collett, 2000; Fry & Wehner, 2002; Menzel et al., 1997), which is quite elementary compared to the cognitive and behavioural capacities of many vertebrates, nevertheless displays its sophistication through the genus's ability to survive in a world multiple times larger and complex than itself.

Despite this sophistication, there are a relatively limited number of neurons in a bee's brain (roughly only 950,000; Menzel, 1990). Even with the ever-increasing sophistication of human technology, it is still a mystery as to how a bee's brain forms a memory for a vector, a landmark, etc. For instance, how did the wasp observed by

Tinbergen and Kruyt (Tinbergen, 1969) form a memory for the pinecone ring? Similarly, how did bees in this experiment form their memories of the surroundings? As mentioned earlier, one theory is that bees take multiple snapshots and then engage in image matching between their percepts and these templates. What these snapshots may look like, at least to a human, has not been substantiated. However, it is thought that for reasons of efficiency and capability from a bee's perspective, these snapshots may consist of a series of organized edges and contrasts perceived by the bee, rather than a complete, coloured replica of the surround. (And in this respect the term "snapshot" may be misleading.) On another tack, Menzel (2001) has found evidence for the role of protein synthesis in bee memory; yet any relationship between snapshot-taking and protein formation has yet to be clarified.

One fruitful avenue for uncovering the mysteries of bee memory is neural networking and computer simulation (Churchland & Seinowski, 1994). These methods allow one to determine whether a bee's brain is capable of what is hypothesized; for example, is a bee neurologically capable of taking "snapshots" of its environment? Is their brain capable of giving more priority to proximal landmarks? More specifically, it allows one to explore how motor output is produced from sensory input, and may produce results and predictions not possible from biological and behavioural studies by themselves (Inoue, Kashimori, Yang, Liu, Hoshino, & Kambara, 2001; Masson & Linster, 1995). For instance, Möller, Mavis, and Lambrinos (1999) designed a neural networking model showing that the insect brain is capable of "snapshot matching," while also suggesting that the periphery of the visual system might be the seat of landmark navigation. In addition, Krackauer (1995) demonstrated by connectionist modeling that the larger a bee's "snapshot library," the more accurate the bee's search behaviour. Of course, neural networking cannot stand alone; behavioural and physiological studies in conjunction with this modeling would give a more complete picture of the memory system (Masson & Linster, 1995).

One point that theorists should keep in mind for future research is that a bee's experience is not simply visual. As suggested from the account of scent marking in this thesis as well as previous research (e.g., Bitterman, 1996), olfactory memories can be formed as well. Perhaps even tactile memories are possible. The door is wide open for exploration of the interactions of various forms of sensory memories, during spatial tasks or otherwise. Given that computers and other forms of technology at our disposal now allow us to see what the unaided human eye cannot, combining these with behavioural and physiological methods should indeed lead to very exciting discoveries regarding bee memory.

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APPENDIX A

Tables 1-9 Presenting Individual Data for All Experiments

Table 1.

Individual Data for Experiment 1

Bee	Colony	Nature of Flowers[a]	Assignment of S+[b]	Choice[c]	Response Type[d]
1	1	1	1	0	1
2	2	1	2	1	1
3	3	1	3	0	1
4	1	1	1	1	1
5	2	1	2	1	1
6	3	1	3	1	1
7	1	1	1	1	1
8	2	1	2	1	1
9	3	1	3	1	1
10	1	1	1	1	1
11	2	1	2	1	0
12	3	1	3	1	1
13	1	1	1	1	1
14	2	1	2	1	1
15	3	1	3	1	1
16	1	1	1	1	0
17	2	1	2	0	1
18	3	1	3	1	1
19	1	1	1	1	1
20	2	1	2	1	1
21	3	1	3	1	1
22	1	1	1	1	1
23	2	1	2	1	1
24	3	1	3	1	1
25	1	2	1	1	1
26	1	2	1	0	1
27	2	2	2	0	1
28	2	2	2	1	0
29	3	2	3	1	0
30	3	2	3	1	1
31	1	2	1	1	1
32	1	2	1	0	1
33	2	2	2	1	0
34	2	2	2	0	1
35	3	2	3	1	0
36	3	2	3	1	1
37	1	2	1	1	0
38	1	2	1	1	1
39	2	2	2	1	0

Table continues

Bee	Colony	Nature of Flowers[a]	Assignment of S+[b]	Choice[c]	Response Type[d]
40	2	2	2	0	0
41	3	2	3	1	0
42	3	2	3	1	0
43	1	2	1	1	1
44	1	2	1	0	1
45	2	2	2	1	0
46	2	2	2	1	0
47	2	2	3	1	0
48	3	2	3	1	0
49	1	3	1	1	1
50	2	3	2	1	1
51	3	3	3	0	0
52	1	3	1	1	0
53	2	3	2	1	0
54	3	3	3	1	0
55	1	3	1	1	0
56	2	3	2	1	0
57	3	3	3	1	0
58	1	3	1	0	0
59	2	3	2	1	0
60	3	3	3	1	0
61	1	3	1	0	0
62	2	3	2	0	0
63	3	3	3	1	0
64	1	3	1	1	0
65	2	3	2	1	0
66	3	3	3	1	0
67	1	3	1	1	0
68	2	3	2	0	0
69	3	3	3	1	1
70	1	3	1	0	0
71	2	3	2	1	0
72	3	3	3	1	0

[a] 1 = old cover, 2 = old cover moved, 3 = new cover. [b] 1 = left flower, 2 = middle flower, 3 = right flower. [c] 1 = absolute position of S+ chosen, 0 = absolute position of S+ not chosen. [d] 1 = probe, 0 = not probe.

Table 2.

Individual Data for Experiment 2

Bee	Colony	Nature of Flowers[a]	Assignment of S+[b]	Proportion of Rewarding Visits	Choice[c]	Response Type[d]
1	2	1	3	.39	1	1
2	2	2	3	1.00	0	0
3	2	1	3	.86	1	1
4	2	2	3	.83	0	0
5	1	1	2	1.00	1	1
6	2	2	2	.50	1	0
7	1	1	2	.71	1	1
8	2	2	2	.60	1	0
9	1	1	1	.79	1	1
10	1	2	1	.56	0	1
11	1	1	1	.68	1	1
12	1	2	1	.58	0	0
13	2	1	3	.92	0	1
14	2	2	3	.33	1	0
15	2	1	3	.79	0	1
16	2	2	3	.60	0	0
17	1	1	2	.57	1	1
18	2	2	2	.59	0	0
19	1	1	2	.55	1	1
20	2	2	2	.33	1	0
21	1	1	1	.54	1	1
22	1	2	1	.90	1	1
23	1	1	1	.49	1	1
24	1	2	1	.63	1	1

[a] 1 = old cover, 2 = new cover. [b] 1 = left flower, 2 = middle flower, 3 = right flower. [c] 1 = absolute position of S+ chosen, 0 = absolute position of S+ not chosen. [d] 1 = probe, 0 = not probe.

Table 3.

Individual Data for Experiment 1 of the Journal Manuscript, Condition 1: Old Covers Unmoved, Flowers Unmoved

Bee	Assignment of S+[a]	Flowers Move?[b]	Nature of Flowers[c]	Proportion of Rewarding Visits	Choice[d]	Response Type[e]
1	2	0	1	.68	1	1
2	2	0	1	.59	1	1
3	2	0	1	.47	1	1
4	2	0	1	.52	1	1
5	2	0	1	.62	1	1
6	2	0	1	.81	1	1
7	2	0	1	.90	1	1
8	2	0	1	.69	0	1
9	2	0	1	.78	1	1
10	2	0	1	.81	1	1
11	2	0	1	.73	1	1
12	2	0	1	.71	1	1
13	1	0	1	.75	1	1
14	1	0	1	.68	1	0
15	1	0	1	1.00	1	1
16	1	0	1	1.00	1	1
17	1	0	1	.84	1	0
18	1	0	1	.67	0	0
19	1	0	1	.74	1	1
20	1	0	1	.86	1	1
21	1	0	1	.62	1	1
22	1	0	1	.70	1	1
23	1	0	1	.70	1	1
24	1	0	1	.65	0	1

[a]1 = left flower, 2 = right flower. [b]1 = flower moved, 0 = flower unmoved. [c]1 = old cover unmoved, 2 = old cover moved, 0 = new cover. [d]1 = FAI position of S+ chosen, 0 = FAI position of S+ not chosen. [e]1 = probe, 0 = not probe.

Table 4.

Individual Data for Experiment 1 of the Journal Manuscript, Condition 2: Old Covers Unmoved, Flowers Moved

Bee	Assignment of S+[a]	Flowers Move?[b]	Nature of Flowers[c]	Proportion of Rewarding Visits	Choice[d]	Response Type[e]
25	2	1	1	.54	0	1
26	2	1	1	.60	1	0
27	2	1	1	.92	1	1
28	2	1	1	.71	1	0
29	2	1	1	.59	1	1
30	2	1	1	.43	1	0
31	2	1	1	.96	1	1
32	2	1	1	.70	1	1
33	2	1	1	.73	0	1
34	2	1	1	.62	0	1
35	2	1	1	.50	0	1
36	2	1	1	.70	1	1
37	1	1	1	.57	1	1
38	1	1	1	.81	1	1
39	1	1	1	.57	0	0
40	1	1	1	.64	1	1
41	1	1	1	.64	0	0
42	1	1	1	.58	1	1
43	1	1	1	.66	1	1
44	1	1	1	.62	1	1
45	1	1	1	.74	1	1
46	1	1	1	.80	1	1
47	1	1	1	.60	1	1
48	1	1	1	.62	0	1

[a] 1 = left flower, 2 = right flower. [b] 1 = flower moved, 0 = flower unmoved. [c] 1 = old cover unmoved, 2 = old cover moved, 3 = new cover. [d] 1 = FAI position of S+ chosen, 0 = FAI position of S+ not chosen. [e] 1 = probe, 0 = not probe.

Table 5.

Individual Data for Experiment 1 of the Journal Manuscript, Condition 3: Old Covers Moved, Flowers Unmoved

Bee	Assignment of S+[a]	Flowers Move?[b]	Nature of Flowers[c]	Proportion of Rewarding Visits	Choice[d]	Response Type[e]
49	2	0	2	.79	1	1
50	2	0	2	.85	0	0
51	2	0	2	.72	1	1
52	2	0	2	.55	1	0
53	2	0	2	.60	1	1
54	2	0	2	.80	1	0
55	2	0	2	.63	1	1
56	2	0	2	.67	1	1
57	2	0	2	.73	1	1
58	2	0	2	.77	1	1
59	2	0	2	.90	1	1
60	2	0	2	.56	1	1
61	1	0	2	.79	1	1
62	1	0	2	.89	1	1
63	1	0	2	.74	1	0
64	1	0	2	.65	1	1
65	1	0	2	.81	1	0
66	1	0	2	.69	1	1
67	1	0	2	.87	1	1
68	1	0	2	.74	1	1
69	1	0	2	.89	1	1
70	1	0	2	.91	1	1
71	1	0	2	.67	1	1
72	1	0	2	.76	1	1

[a] 1 = left flower, 2 = right flower. [b] 1 = flower moved, 0 = flower unmoved. [c] 1 = old cover unmoved, 2 = old cover moved, 3 = new cover. [d] 1 = FAI position of S+ chosen, 0 = FAI position of S+ not chosen. [e] 1 = probe, 0 = not probe.

Table 6.

Individual Data for Experiment 1 of the Journal Manuscript, Condition 4: Old Covers Moved, Flowers Moved

Bee	Assignment of S+[a]	Flowers Move?[b]	Nature of Flowers[c]	Proportion of Rewarding Visits	Choice[d]	Response Type[e]
73	2	1	2	.57	1	1
74	2	1	2	.68	1	0
75	2	1	2	.77	1	1
76	2	1	2	.72	1	1
77	2	1	2	.79	0	0
78	2	1	2	.76	0	1
79	2	1	2	.71	1	1
80	2	1	2	.77	1	1
81	2	1	2	.31	0	1
82	2	1	2	.79	1	1
83	2	1	2	.56	1	1
84	2	1	2	.70	0	1
85	1	1	2	.74	1	1
86	1	1	2	.77	1	0
87	1	1	2	.85	1	0
88	1	1	2	.53	0	1
89	1	1	2	.50	1	0
90	1	1	2	.70	1	1
91	1	1	2	.85	0	1
92	1	1	2	.83	1	1
93	1	1	2	.74	1	0
94	1	1	2	.79	1	0
95	1	1	2	.62	1	0
96	1	1	2	.54	1	0

[a] 1 = left flower, 2 = right flower. [b] 1 = flower moved, 0 = flower unmoved. [c] 1 = old cover unmoved, 2 = old cover moved, 3 = new cover. [d] 1 = FAI position of S+ chosen, 0 = FAI position of S+ not chosen. [e] 1 = probe, 0 = not probe.

Table 7.

Individual Data for Experiment 1 of the Journal Manuscript, Condition 5: New Covers, Flowers Unmoved

Bee	Assignment of S+[a]	Flowers Move?[b]	Nature of Flowers[c]	Proportion of Rewarding Visits	Choice[d]	Response Type[e]
97	1	0	3	.77	1	0
98	1	0	3	.78	1	1
99	1	0	3	.59	1	0
100	1	0	3	.77	1	1
101	1	0	3	.81	1	1
102	1	0	3	.57	1	1
103	1	0	3	.76	1	0
104	1	0	3	.70	1	0
105	1	0	3	.62	1	0
106	1	0	3	.41	1	0
107	1	0	3	.73	1	0
108	1	0	3	.75	1	1
109	2	0	3	.88	1	1
110	2	0	3	.49	1	1
111	2	0	3	.69	1	1
112	2	0	3	.80	1	0
113	2	0	3	.80	1	0
114	2	0	3	.77	1	1
115	2	0	3	.81	1	0
116	2	0	3	.85	1	1
117	2	0	3	.74	1	1
118	2	0	3	.74	1	1
119	2	0	3	.67	1	0
120	2	0	3	.78	1	1

[a] 1 = left flower, 2 = right flower. [b] 1 = flower moved, 0 = flower unmoved. [c] 1 = old cover unmoved, 2 = old cover moved, 3 = new cover. [d] 1 = FAI position of S+ chosen, 0 = FAI position of S+ not chosen. [e] 1 = probe, 0 = not probe.

Table 8.

Individual Data for Experiment 1 of the Journal Manuscript, Condition 6: New Covers, Flower Moved

Bee	Assignment of S+[a]	Flowers Move?[b]	Nature of Flowers[c]	Proportion of Rewarding Visits	Choice[d]	Response Type[e]
121	1	1	3	.54	0	1
122	1	1	3	.80	1	0
123	1	1	3	.93	1	0
124	1	1	3	.79	1	1
125	1	1	3	.87	1	0
126	1	1	3	.72	1	1
127	1	1	3	.76	1	1
128	1	1	3	.46	1	1
129	1	1	3	.63	1	1
130	1	1	3	.76	0	0
131	1	1	3	.77	1	0
132	1	1	3	.54	0	1
133	2	1	3	.74	1	1
134	2	1	3	.73	1	0
135	2	1	3	.59	1	1
136	2	1	3	.85	0	1
137	2	1	3	.87	1	0
138	2	1	3	.80	1	1
139	2	1	3	.86	1	1
140	2	1	3	.69	1	0
141	2	1	3	.77	0	0
142	2	1	3	.61	1	0
143	2	1	3	.59	1	1
144	2	1	3	.85	1	1

[a] 1 = left flower, 2 = right flower. [b] 1 = flower moved, 0 = flower unmoved. [c] 1 = old cover unmoved, 2 = old cover moved 3 = new cover. [d] 1 =FAI position of S+ chosen, 0 = FAI position of S+ not chosen. [e] 1 = probe, 0 = not probe.

Table 9.

Individual Data for Experiment 2 of the Journal Manuscript

Bee	Colony	Condition[a]	Colony Position in Training[b]	Assignment of S+[b]	Proportion of Rewarding Visits	Colony Position in Testing[b]	Choice[c]	Response Type[d]
1	2	1	3	3	0.48	3	2	1
2	2	2	3	3	0.61	2	1	1
3	2	2	3	3	0.42	1	2	1
4	2	1	3	3	0.36	3	2	1
5	2	2	3	3	0.39	2	2	1
6	2	2	3	3	0.39	1	2	1
7	2	1	3	2	0.47	3	2	1
8	2	2	3	2	0.64	2	2	1
9	2	2	3	2	0.52	1	2	1
10	2	1	3	2	0.50	3	2	1
11	2	2	3	2	0.61	2	2	1
12	2	2	3	2	0.38	1	2	1
13	2	1	3	1	0.35	3	2	1
14	2	2	3	1	0.53	2	2	1
15	2	1	3	1	0.42	3	1	1
16	2	2	3	1	0.29	2	2	1
17	2	1	2	3	0.60	2	2	1
18	2	2	2	3	0.52	3	2	1
19	2	2	2	3	0.30	1	2	1

Table continues

Bee	Colony	Condition[a]	Colony Position in Training[b]	Assignment of S+[b]	Proportion of Rewarding Visits	Colony Position in Testing[b]	Choice[c]	Response Type[d]
20	2	1	2	3	0.31	2	1	1
21	2	2	2	3	0.51	3	2	1
22	2	2	2	3	0.75	1	1	1
23	2	1	2	2	0.43	2	2	1
24	2	2	2	2	0.50	3	1	1
25	2	2	2	2	0.63	1	2	1
26	2	1	2	2	0.41	2	2	1
27	2	2	2	2	0.45	3	2	1
28	2	2	2	2	0.39	1	1	1
29	1	1	2	1	0.50	2	1	1
30	1	2	2	1	0.69	3	2	1
31	1	2	2	1	0.34	1	2	2
32	1	1	2	1	0.55	2	1	0
33	1	2	2	1	0.45	3	2	1
34	1	2	2	1	0.58	1	2	1
35	1	1	1	3	0.54	1	2	1
36	1	2	1	3	0.51	2	1	1
37	1	1	1	3	0.71	1	2	1
38	1	2	1	3	0.70	2	2	1
39	1	1	1	2	0.48	1	1	1
40	1	2	1	2	0.70	3	2	1

Table continues

Bee	Colony	Condition[a]	Colony Position in Training[b]	Assignment of S+[b]	Proportion of Rewarding Visits	Colony Position in Testing[b]	Choice[c]	Response Type[d]
41	1	2	1	2	0.76	2	2	1
42	1	1	1	2	0.74	1	2	2
43	1	2	1	2	0.55	3	1	0
44	1	2	1	2	0.53	2	2	1
45	1	1	1	1	0.42	1	2	1
46	1	2	1	1	0.69	3	2	1
47	1	2	1	1	0.67	2	2	1
48	1	1	1	1	0.86	1	1	1
49	1	2	1	1	0.73	3	2	1
50	1	2	1	1	0.57	2	2	1

[a] 1 = control, 2 = test. [b] 1 = left, 2 = middle, 3 = right. [c] 2 = allocentric, 1 = not allocentric.

[d] 2 = probe, 1 = land, 0 = no response.

APPENDIX B

Table Presenting the Frequencies for Second and Third Test Choices and Probe Response for Bees in Experiment 2 of Chapter Three

Table 1.

Observed Frequencies of Second and Third Test Flower Choices, Expected Values According to Chance, and Number of Probe Responses for Experiment 2 of the Journal Manuscript

	S+ Position	Other Position	# Probes
	Control Group: Second Choice		
Observed	13*	2	2
Expected	7.5	7.5	
	Control Group: Third Choice		
Observed	12*	0	0
Expected	6	6	

	FAI Position	Vector Memory Position	# Probes
	Test Group: Second Choice		
Observed	21	7	2
Expected	14	14	

Table continues

	FAI Position	Vector Memory Position	# Probes
	Test Group: Third Choice		
Observed	17	6	2
Expected	11.5	11.5	
	Test Group, Middle Flower S+: Second Choice		
Observed	9	1	0
Expected	5	5	
	Test Group, Middle Flower S+: Third Choice		
Observed	9	0	1
Expected	4.5	4.5	

Note. For the Control Group, S+ position can be chosen using FAI (Flower Array Independent) information or vector memory.

APPENDIX C

Sample Output for GLIM Analysis: Experiment 2 of Chapter Two, All Conditions.

```
$c 2 colonies, 3 training flowers, old (1) new (2)$
$c num rew visits, num total visits, prop rew vis,
$c type of response 1=probe, 2=land, 3=approach$
$data bee col nold rflow rew vis prop choice behav$
$dinput 11$
File name? Danex2.txt
Bee Colony N/O  Rflow  Rew  Visits  Prop  Choice Behav
1     2     1     3     23    59    0.39    1     1
2     2     2     3     2     2     1       0     2
3     2     1     3     19    22    0.86    1     1
4     2     2     3     25    30    0.83    0     2
5     1     1     2     13    13    1       1     1
6     2     2     2     8     16    0.5     1     2
7     1     1     2     59    83    0.71    1     1
8     2     2     2     12    20    0.6     1     2
9     1     1     1     81    103   0.79    1     1
10    1     2     1     71    127   0.56    0     1
11    1     1     1     50    73    0.68    1     1
12    1     2     1     57    98    0.58    0     2
13    2     1     3     12    13    0.92    0     1
14    2     2     3     7     21    0.33    1     2
15    2     1     3     15    19    0.79    0     1
16    2     2     3     9     15    0.6     0     2
17    1     1     2     8     14    0.57    1     1
18    2     2     2     22    37    0.59    0     2
19    1     1     2     31    56    0.55    1     1
20    2     2     2     3     9     0.33    1     2
21    1     1     1     51    95    0.54    1     1
22    1     2     1     35    39    0.9     1     1
23    1     1     1     37    76    0.49    1     1
24    1     2     1     15    24    0.63    1     1
$fac col 2 nold 2 rflower 3 behav 3$
$yvar choice$
$cal n=1$
$c binomial denominator n=1 choice per bee$
$tab the choice mean for nold$
 NOLD    1      2
 MEAN  0.8333  0.5000
$tab the vis mean for nold$
 NOLD    1     2
 MEAN  52.17  36.50
$tab the rew mean for nold$
 NOLD    1     2
 MEAN  33.25  22.17
```

```
$tab the prop mean for nold$
 NOLD    1      2
 MEAN  0.6908  0.6208
$tab the choice mean for nold;rflow into wmean by w
$tprint(s=1) wmean wnold;wrflow$
        +---------------------------+
  WRFLOW |  1.000    2.000    3.000 |
   WNOLD |                          |
+--------+---------------------------+
|      1 |  1.0000   1.0000   0.5000 |
|      2 |  0.5000   0.7500   0.2500 |
+--------+---------------------------+
$err b n$
$fit:+col:+vis:+rew:+prop:+rflow:+nold:+nold.rflow$
scaled deviance =  30.553 at cycle 3
   residual df =  23

scaled deviance =  27.449 (change =   -3.104) at
cycle 2
   residual df =  22     (change =   -1    )

scaled deviance =  25.100 (change =   -2.349) at
cycle 4
   residual df =  21     (change =   -1    )

scaled deviance =  24.650 (change =  -0.4500) at
cycle 4
   residual df =  20     (change =  -1     )

scaled deviance =  17.886 (change =   -6.765) at
cycle 5
   residual df =  19     (change =   -1    )

scaled deviance =  17.600 (change =  -0.2853) at
cycle 5
   residual df =  18     (change =  -1     )

scaled deviance =   7.4209 (change =   -10.18) at
cycle 7
   residual df =  17      (change =    -1  )

scaled deviance =   7.3984 (change =  -0.02253) at
cycle 10
   residual df =  16      (change =  -1      )
-- (no convergence yet)
$stop$
```